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CONTENTS OF VOL. VIII, No. 1

PAGE

1. The Protection of Meat Commodities against Blowflies. By R. A. WARDLE 1
2. On the Fungus Flora of Glasshouse Water Supplies in Relation to Plant Disease. By W. F. BEWLEY and W. BUDDIN. (With 1 Text-figure) 10
3. Studies in Bacteriosis. V. Further Investigation of a suggested Bacteriolytic Action in *Protea cynaroïdes* affected with the Leaf-spot Disease. By SYDNEY G. PAINE and EMILY M. BERRIDGE. (With 1 Text-figure) 20
4. The Killing of Botrytis Spores by Phenol. By J. HENDERSON SMITH, M.B., Ch.B. (With 11 Text-figures) 27
5. Biological Studies of *Aphis rumicis* Linn. 1746. III. By J. DAVIDSON, D.Sc. (With 1 Text-figure) 51
6. Some Relationships of Economic Biology. By SIR DAVID PRAIN, C.M.G., C.I.E., LL.D., F.R.S. 66
7. Proceedings of the Association of Economic Biologists . . . 77

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THE PROTECTION OF MEAT COMMODITIES AGAINST BLOWFLIES

BY R. A. WARDLE.

(*University of Manchester.*)

A SURVEY of the literature concerning the economic aspect of the Muscid genera comprised in the popular term "blowfly" will indicate that, although much work has been carried out with respect to the prevention of oviposition upon living animals, and although several recent papers have dealt very fully with the problem of trapping or poisoning Muscid flies in general, yet the question of directly preventing oviposition upon foodstuffs has been neglected somewhat and its importance overlooked.

In Manchester, the alternate periods of fine rain and sunshine that constitute a normal summer are especially favourable to blowflies. Swarms of such flies are particularly noticeable in those shops—butchers, fishmongers, fruiterers and the market stalls—where, from motives of ventilation, the window space is not enclosed by glass. In such shops the usual methods of fly-prevention are impracticable. Poison bait traps do not enhance the appearance of a window containing food commodities. Screens of mosquito netting impede the circulation of air, so necessary in this type of shop, and becoming quickly soiled by juices of meat or fruit, obscure the view of the purchaser.

Attempts at fly-exclusion are therefore almost non-existent. The butcher will assert that his meat rarely becomes flyblown, a statement based generally upon the popular belief that only tainted meat is attacked by blowflies, but supported somewhat as a fact by the custom of keeping meat overnight in a refrigerator and placing it upon the boards of the open window in a slightly chilled and coagulated condition. The fishmonger is protected to some extent by the use of ice slabs and the hose-pipe. The fruiterer is frankly indifferent.

CONDITIONS CONDUCIVE TO OVIPOSITION.

The factors which affect oviposition fall readily into two categories: (*a*) nature of the foodstuffs attacked, (*b*) meteorological conditions.

The range of substances attractive to blowflies is wide, comprising

particularly flesh foods, ripe or over-ripe fruits, sugars (fructose, honey, molasses), faeces, stale urine, etc., but the range of substances upon which such flies will lay eggs is limited. My own experiments, confined solely to substances exposed for sale as food in open shops, and in a condition fit for human consumption, indicated the following order of preference: raw liver, raw sheep kidney, raw lean mutton, raw lean beef, beef cooked but underdone, bacon (mild cured).

On the other hand, food commodities apparently inconducive to oviposition comprised: chilled meat, well-cooked meat, over-ripe fruit, preserved meats (sausages, tinned meats, salt bacon), tripe as prepared for retail sale, fresh fish, animal fats, cheese, shellfish. Fish was only attacked when far too stale for human food.

That is to say, the substances upon which the blowfly prefers to oviposit are those which contain animal proteins, in particular those proteins known as *albumins* and *globulins*.

Albuminoid substances are not attractive, attempts to induce oviposition upon such substances as gelatin, ox-eye, fish-skin, rabbit-skin, etc., being unsuccessful.

It is not sufficient that the foodstuff be moist. It would seem necessary that the protein content has not been coagulated by heat nor washed out by water or salt solution. Slices of raw liver, for example, are highly attractive to blowflies; if the liver, however, be well washed in water or soaked in weak brine, so as to clear away temporarily the exuding blood and muscle plasma, the power of attraction is lessened; if the slice be singed superficially with a Bunsen flame or allowed to desiccate naturally in the sunshine, the power of attraction goes, however moist the surface be kept afterwards. Freshly-cooked meat, however moist, does not readily attract blowflies. Again, tripe is always extremely moist when fresh, but the windows of shops containing this commodity are conspicuously free from blowflies; repeated attempts to induce *Calliphora* to oviposit upon tripe were unsuccessful, nor could larvae be prevailed upon to feed on this substance.

The stimulus to oviposition, whether olfactory or gustatory, would seem to exist in the exuding juices, blood and muscle plasma, of the substance attacked, and particularly does the source of attraction appear to be the muscle plasma, for blood, whether fresh or putrid, seems quite unattractive, an observation previously noted by Miss Lodge (4); there is no doubt, also, that blowflies are more strongly attracted by putrefying substances than by fresh, and Miss Lodge recommends as the most attractive trap-baits: liver + maggots, brain + maggots, fish + maggots,

hard-boiled egg + maggots, that is to say, animal proteins in the early stages of putrefaction brought about by the digestive juices of the maggots.

If the digestive ferment of the larva be an enzyme analogous to pepsin, that is to say be proteoclastic and not lipoclastic, a conclusion warranted by its inability to hydrolyse fats, then the only products from the hydrolysis of muscle plasma would be Secondary Protein Derivatives, particularly proteoses and peptones. Anaerobic bacterial action commences early, however, and even in freshly-killed meat the breaking down of the peptones into amino-acids and into later putrefaction bases is slowly taking place. In fresh muscle attacked by maggots, the proteoclastic enzyme *protase*, occurring naturally in muscle, is accelerated by the enzyme of the maggot, and the hydrolysis of the proteins to amino-acids and the decomposition of these into putrefaction bases are comparatively rapid, so that in the broth produced by the action of blowfly larvae upon meat, such bases as *indole*, *skatole*, *methylamine*, *putrescine*, *methylguanidine*, would occur early.

That blowflies are chemotropic to such bases seems indisputable when the attraction of such substances as faeces, stale urine or putrid yeast is considered; yet Miss Lodge found hard-boiled egg plus methyl indole, or fresh meat plus skatole, to be quite unattractive, and no special attraction was evinced for *trimethylamine*, *tyrosine*, *guanine*, and *dimethylamine*.

It would seem doubtful at any rate whether the power of attraction of such bases is sufficient to induce the fly to oviposit. My own attempts to bring about oviposition upon human faeces and putrid yeast were unsuccessful. Howlett, however, has induced a species of *Sarcophaga* to oviposit upon skatole, and Mellor(5) states that "*C. erythrocephala* laid eggs upon the baits—human faeces, plums, milk and sheep's noses—which were placed together in the same receptacle."

It must be noted too that blowflies will readily oviposit upon freshly-dressed meat where the amount of such putrefaction bases must be very slight.

Further experiments may indicate that the stimulus to oviposition must be looked for among the amino-acids, and that in *Calliphora* the stimulus may be chiefly gustatory, and in *Sarcophaga* olfactory, which would account for the difference in response to skatole.

Meteorological factors, if not of such direct importance as the food factors, nevertheless play an important part. According to Miss Lodge an attractive bait placed in the shade attracted no blowflies, although

they had been swarming round it when it was placed in the sun. In my own experiments, the baits were duplicated, one series being placed in the sunniest part of a large garden, another series being placed in the shadiest portion of an adjoining insectary, which, having side walls of large mesh chicken wire, was quite open to flies. Both batches were equally readily infested within a few minutes of exposing, but in few cases did oviposition occur upon the baits exposed to the sunshine, and even then the eggs were few and scattered, whereas the baits in the shade were readily blown.

This apparent discrepancy in observations may be explained by an undoubted difference in response to light between the *Calliphora* type of blowfly and the *Lucilia* type. The flies used by Miss Lodge were chiefly *Lucilia*. Among the flies that visited my baits, *Lucilia* was in the minority. In fact numerous samples taken from various parts of the city all showed a marked predominance of *Calliphora vomitoria*, this fly constituting on the average 75 per cent. of the sample, the remaining percentage being made up almost equally of *Calliphora erythrocephala* and *Lucilia caesar*. In addition to being in the minority, *Lucilia* had also to compete for foothold on the baits not only with *Calliphora* but with hordes of Acalyptrate Muscidae as well.

The difference in response to light has been pointed out by Herms (2): "The more frequent presence of *Calliphora vomitoria* in houses and like situations is due chiefly to a relatively low degree of responsiveness to light, so that odours from darker places may attract it more readily. On the other hand, *Lucilia caesar* is very strongly phototactic and consequently would seek the open, and if by chance it should find its way into darker places, its responsiveness to large luminous areas would soon lead it to escape. It may be assumed that the two species are equally chemotactic, which assumption is justified at least by observation. Since *C. vomitoria* is less strongly phototactic, individuals least so might easily be attracted into fairly dark places and would not soon be compelled to leave, because of their phototropism."

This difference in habit was strikingly observed during the hot August sunshine of 1916 in the trenches outside Delville Wood, on the Somme, where blowflies were abnormally abundant. In the shade afforded by the deeper portion of the trench, round the traverses, any moist patch on the chalk wall would be hidden by a dense indigo-coloured cluster of *Calliphora*, large as a soup plate, unredeemed by the metallic green of *Lucilia*, whereas, where the trench was shallow or blown in, the green shimmer of *Lucilia* was everywhere.

It would appear too that *Lucilia* prefers carrion in bulk and is primarily a fly infesting carcases in the open, thus not having the economic significance of *Calliphora* as regards foodstuffs.

The hygrometric condition of the atmosphere is not without significance. In continuously fine dry weather, oviposition occurred more frequently in the early morning between dawn and 8 a.m., whilst the atmosphere was somewhat moist. A sunny morning following a wet night, or a sunny afternoon following a wet morning, were particularly favourable.

Wind was antagonistic. Few flies were on the wing and the baits became dry and unattractive.

EXPERIMENTS IN PREVENTION.

Screening and trapping, as already pointed out, are not methods readily applicable to the problem in hand. Attention was therefore directed to the question of repellent substances.

These fall into two classes:

(1) Substances directly applicable to foodstuffs.

(2) Substances indirectly applicable to foodstuffs.

The range of repellent substances that can be directly applied is limited. A trial was made with the household remedies usually recommended against flyblow, such as vinegar, onion juice, pepper, salt, tomato juice, etc., and the list was extended by the addition of formalin, boracic acid, and a proprietary article termed Milton, having a slight chlorine odour. Slices of raw kidney or liver were sprayed with or dipped in solutions of these substances and exposed, one batch in the sunshine, one batch in the shade. The results obtained from the baits exposed in the sunshine were of little use as even the controls were rarely blown. Flies fed freely on all the samples but particularly on those treated with vinegar, weak Milton, and borax. The samples treated with concentrated Milton and with $2\frac{1}{2}$ per cent. formalin were avoided when flies were few, when the number of flies increased these samples were attacked also.

The following table is typical of the oviposition results observed in twenty repetitions of the experiment.

The weak solution of Milton would appear to be more repellent than the strong but other experiments did not confirm this. In fact, weak Milton and vinegar seemed slightly attractive. The success of pepper appears due to the drying effect produced upon the surface of the bait. It was not so effective when applied to liver or to very juicy meat. Precipitated chalk gave similar results. Cooper and Walling(3), experi-

menting with a wide range of repellent substances against blowflies, used precipitated chalk as a vehicle for the chemicals. In most cases, the chalk plus chemical conferred immunity during one to four days, complete immunity being obtained by the use of copper carbonate, nitrobenzene, picric acid, creosote, sinapis oil and aniseed oil. It must be noted, however, that precipitated chalk by itself will protect fresh meat so long as it remains unsaturated by exuding juices either of muscle plasma or of putrefaction, and that when chemicals are present that can exert an inhibiting action upon bacterial activity, putrefaction is delayed and the chalk will last a long time before becoming saturated. That is to say, the substances recommended by Cooper and Walling may not necessarily be repellent to the fly, the apparent repellent action being due possibly to the restraining effect exerted by the chemicals upon bacterial activity, and the action of the precipitated chalk in keeping the meat surface dry.

Sliced sheep's kidney placed in insectary at noon	Weather sunny, cool in afternoon. Wet night, fine morning.	
	Dusk	9 a.m. next day
Untreated...	+slightly	+heavily
5 % Milton	–	+slightly
Concentrated Milton	+four scattered eggs	+considerably
Concentrated vinegar	–	+considerably
Formalin 2½ %	–	+slightly
Pepper, dry	–	–
Boracic acid, dry...	+slightly	+considerably
Boracic acid solution, strong ...	+considerably	+heavily
Onion juice	–	+heavily
Lemon juice	+slightly	+heavily
Salt, dry	–	+slightly
Salt, solution	–	+considerably
Formic acid, 5 %	–	+slightly

+signifies blown. –signifies not blown.
"Heavily" signifies three or more clusters of eggs.
"Considerably" signifies one or two clusters of eggs.
"Slightly" signifies scattered eggs, rarely exceeding 12 or 15.

In the case of powdered boracic acid or powdered salt, the drying effect is soon lost owing to the solvent action of the meat juices and no success, as repellents, was observed in my experiments with these substances.

Cooper and Walling, and Miss Lodge, however, assert positively that boracic acid is repellent to blowflies.

Weak formalin was decidedly repellent, but hardly applicable to meat commodities.

The second class of repellent substances, namely such as are indirectly applicable to meat commodities, comprises such substances as have a distinct and penetrating odour, the essential oils, phenols, paraffins, esters, etc. For example, the following list of substances was found by Miss Lodge to be decidedly repellent: *pipendine, oenanthol, xylol, amyl acetate, methyl salicylate, anisole, citral* (strong), *ethyl sulphocyanide, oil of thyme, of cassia, of Java citronella, of palma rosa, of bay, of heliotrope, of lavender, of cinnamon leaf, of cinnamon bark, of sassafras, of cloves, of camphor.*

Cooper and Walling give: *methyl salicylate, p-nitraniline, picric acid, creosote, green oil, boracic acid, mustard oil, sod oil, iodoform, di-methylaniline, quinoline, allyl alcohol, fusel oil, pine oil, alizarine oil, origanum oil, sinapis oil, alloin, saponin, copper carbonate, nitrobenzene, aniseed oil.*

Now a substance may be repellent to a fly attracted to the bait from motives of curiosity or hunger, without being necessarily repellent to a female fly under the far stronger oviposition-impulse. Many of the substances listed above are, further, obviously unsuitable for use in proximity to foodstuffs, and others are too expensive or difficult to procure for the average food vendor.

A selection of substances was made therefore, and, as in practice it would be undesirable to apply them directly to foodstuffs, an indirect method of testing them was adopted. The food samples were placed within cylindrical glass dishes over the opening of which a piece of cotton twine netting was tied, after being smeared with the substance to be tested. The netting was of quarter-inch diamond mesh, wide enough to allow the largest *Calliphora* to slip in and out of the dish; as the dishes were of glass, equal illumination of both sides of the netting was obtained and the possibility of the mesh acting as a mechanical obstacle to the ingress of the flies thus excluded. It has been asserted by Spence (1) that flies can be prevented from entering a room through open doors and windows by covering these openings with wide-mesh netting, even herring netting, provided there is no source of illumination behind the door or window. In my later experiments, however, a box of three feet cubic dimensions, lined with black cloth and completely closed except for a narrow slit, was readily entered by large numbers of *Calliphora*, and was also entered quite easily when one side was removed and replaced by black netting.

To perfume the netting, it was found quite sufficient to moisten the palms of the hands and to roll the netting between them. Substances selected were the more easily obtainable essential oils—cloves, citronella,

cinnamon, aniseed, etc. In addition, formic acid, picric acid, boracic acid, and nitrobenzene were tried. Phenolic and paraffin compounds were not tried as their use in proximity to foodstuffs is scarcely practicable.

The following table is typical of some twenty repetitions of the experiments.

Sliced ox liver placed in insectary at noon	Hot afternoon, rain during night, fine hot day succeeding	
	Dusk	Noon next day
Untreated ...	+ heavily	+ heavily
Oil of cloves ...	+ considerably	+ considerably
„ aniseed ...	–	–
„ eucalyptus	–	+ considerably
„ almonds...	+ heavily	+ heavily
„ citronella	+ considerably	+ considerably
„ cinnamon	+ considerably	+ considerably
Formic acid ...	–	+ considerably
Boracic acid ...	+ considerably	+ considerably
Picric acid ...	+ heavily	+ heavily
Nitrobenzene ...	+ considerably	+ considerably

In most cases, the majority of the samples were blown within six hours, if weather conditions were suitable. Samples protected with eucalyptus oil, formic acid and sometimes clove oil, remained untouched for twelve hours, but were generally attacked after that interval.

Similar results were obtained when the baits were placed in a hothouse, average temperature 78° F., into which blowflies could readily enter.

The trials with aniseed oil were repeated on a larger scale. A miniature market stall was erected. It was divided into two halves by a partition. One half was enclosed on all sides, except the mesh side, by dark material, so as to form a semi-dark chamber; the other half was similarly enclosed by white mosquito netting so as to be quite exposed to sunlight. The cotton netting, after being smeared with oil, was hung loosely upon protruding tacks. A variety of baits was placed within the compartments. As before, the baits placed within the light compartment, though visited by clouds of blowflies, were rarely blown.

In no case were the baits that were protected with aniseed oil blown within twenty-four hours.

Various types of netting were tried but in no case was greater success obtained than with the quarter-inch mesh. The advantage of cotton netting, apart from the fact that it is cheaper than netting made from other material, costing from sixpence to eighteen-pence per square yard

according to thickness of thread, is that it absorbs the oil readily, and, owing to its diamond pattern, is fairly elastic. The oil is fairly expensive if purchased from the retail pharmacist, but can be obtained from W. J. Bush et Cie, Grasse, Alpes Maritimes, or from their Hackney branch, at 3*s*. 9*d*. per lb. or at a lower price for quantity. The exact specification is Oil Aniseed, China Star. Oil of sweet fennel would be probably just as effective in spite of its lower percentage of anethole, but costs 10*s*. per lb. A pound of the oil goes a long way, however, if applied in the manner described.

There would seem little advantage to be gained by diluting with methylated spirits or emulsifying with soap solution. Hasty and interrupted experiments seemed to indicate that the efficiency of emulsions of the oil varies directly in proportion to the percentage of oil present. Thus a 50 per cent. emulsion was not effective after twelve hours, a 25 per cent. emulsion not effective beyond six hours, and an emulsion containing less than 10 per cent. of oil not reliable at all. However, accurate work is needed to clear up this point.

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ON THE FUNGUS FLORA OF GLASSHOUSE WATER SUPPLIES IN RELATION TO PLANT DISEASE[1]

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(With 1 Text-figure.)

It has long been recognised that the elimination of infection centres is an important factor in the control of disease, but while the intimate relation between polluted water and epidemics of human diseases is now common knowledge, little attention has yet been given to the corresponding relation between water contaminations and plant diseases. Attention was drawn to this latter problem by a severe epidemic of "damping-off" in tomato seedlings in the early part of 1919 and preliminary experiments showed that the causal organism, *Phytophthora cryptogea* Pethybr. & L., was carried by the water used in cultivation. Later in the year the same fact was proved in the case of a severe attack of "black-eye" rot of tomatoes caused by *Phytophthora parasitica* Dastur. It was evident that a centre of infection existed in the water supply and this appeared of sufficient importance to justify further study.

Sources of water.

The water necessary for glasshouse cultivation is pumped from a well or other source of supply into a galvanised iron or painted steel tank placed at from 20 to 30 feet above ground. Here it is stored for a varying period and thence conveyed direct to the house by means of piping and hoses. In some nurseries covered tanks are used, but this is by no means a constant practice. Uncovered tanks are open to contamination by air-borne bacteria and fungus spores, as well as by debris dropped by birds, which go there to drink. The sources from which the water is drawn in the Lea Valley are the Water Company's supply,

[1] A grant in aid of publication has been received for this communication.

deep bricked artesian wells, shallow surface wells, brooks and ponds. The position of the wells or other source of supply with regard to the actual houses varies considerably, some being placed in a central position surrounded by glasshouses while others are at various distances away. Analyses have been made of water from wells, etc., of different types and position in order to ascertain how far these factors affect its purity as regards contamination with pathogenic micro-organisms.

Experimental.

A reliable method of sampling was devised by filtering large volumes of water through a filter prepared in the following manner, and examining

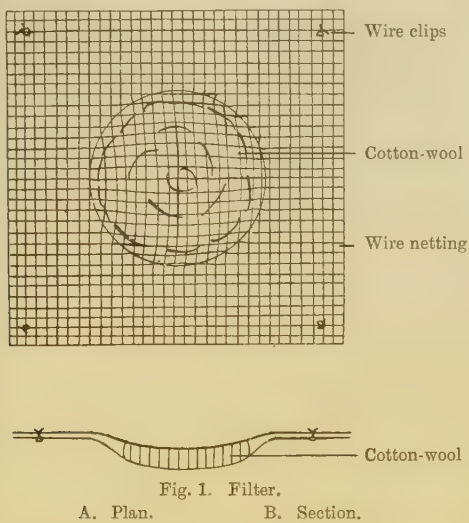

Fig. 1. Filter.
A. Plan. B. Section.

the debris collected. A piece of wire netting, eight inches square, with four meshes to the inch, was cupped in the centre, making a circular depression four inches in diameter and three quarters of an inch deep, Fig. 1. A layer of absorbent wool about a fifth of an inch thick was placed in the circular depression and held in place by another piece of netting similar to the first, but having the central depression only half an inch deep. The two pieces of netting were firmly fixed together at the four corners by means of copper wire, the filter wrapped in brown paper and sterilised. To obtain the necessary sample, the sterile filter was secured over the outlet hole of the tank when the latter was empty,

and the tank then filled and approximately 2,000 gallons of water allowed to pass through the filter. The outlets vary from four to eight inches in diameter and much of the water passed through the netting beyond the wool. This was necessary to prevent the filter from bending, but sufficient water passed through the cotton-wool to yield an adequate supply of bacteria and fungus spores. When the tank was empty, the filter was removed, wrapped in a new piece of sterile brown paper and taken to the laboratory for examination. In sampling the water direct from the main, from brooks and ponds, the filter was fixed under the inlet pipe which fills the tank, protection from aerial infection being provided by tying a piece of sterile paper above the pipe and filter. The cotton-wool plug and the debris held by it was transferred as quickly as possible to 500 c.c. of physiological salt solution, shaken for five minutes and dilutions of 1/25 and 1/250 then made in further salt solutions. 1 c.c. was removed from each dilution and plated upon the following media: (1) Quaker Oat Agar (Clinton)(2), (2) Modified Egg-Albumen Agar (Waksman)(6), (3) Cook's Agar No. II (Cook)(1), (4) Potato Agar.

Potato agar yields a rapid luxuriant growth suitable for preliminary examination, but the plates are often too badly contaminated with bacteria to allow the slow-growing fungi to develop. Quaker Oat agar is specially suited for determining the presence of the various species of *Phytophthora* but if any of the Mucorales are present they develop too rapidly and suppress the development of the more slowly-growing species. Cook's agar proved to be an excellent medium for the work. The various organisms, except the species of *Phytophthora*, develop rapidly and are readily distinguished: pycnidial forms are specially well shown. The Mucorales grow rapidly and if large numbers are present they tend to obscure the other fungi. Modified egg-albumen agar produces a slow growth of all the fungi. It is suitable for careful study and isolation of the various colonies. The plating of the water samples was carried out in the usual manner, special care being taken to cool the melted agars where possible to 45° C., because of the delicacy of some fungus spores. All plates were incubated at 22° C. and examined every 24 hours, only the actual parasites being identified. Doubtful organisms were isolated in pure culture and tested for pathogenicity by inoculation. By utilising all four media a reasonably accurate qualitative estimate of the fungus flora of the water sample was obtained.

In Table I representative water samples are given, and a careful examination appears to justify the conclusion that the water supply may be so seriously contaminated as to constitute an important source

Table I.

Source of water sample arranged in order of purity	Number of samples tested	No. of times each fungus found in total number of samples: Botrytis cinerea	Cladosporium cucumerinum	C. fulvum	C. herbarum	Fusarium sp.	Macrosporium solani	Phytophthora cryptogea	Ph. parasitica	Phoma sp.	Rhizoctonia solani	Rhizopus nigricans	Verticillium albo atrum	Average number of fungi per sample	Remarks
Water Company, direct from main	4	.	.	.	1	.	.	.	.	4	.	.	.	1·25	These records may be actually contaminations in the water supply, but are more likely to be nozzle contaminations
Water Company, covered tank ...	4	1	.	.	1	.	.	.	.	4	.	.	.	1·50	As above
Deep artesian well	2	.	.	.	1	1	.	.	.	1	.	.	.	1·50	These contaminations are possibly from the air, during filtration
Deep well	6	.	.	1	6	4	4	.	.	3	3	2	1	4·00	Faulty brickwork allows surface contamination
Water Company, uncovered tank	6	4	.	3	6	1	2	.	.	6	.	4	2	4·70	
Brook after cleaning	1	1	.	.	1	1	.	.	.	.	.	1	1	5·00	Vegetation was removed and banks cut clean
Shallow well clear of houses ...	7	2	.	4	7	7	.	2	2	4	2	7	.	5·30	
Shallow well surrounded by houses	6	4	1	1	6	6	3	2	1	2	3	5	5	6·50	
Shallow well surrounded by houses; high organic content	2	2	.	.	2	2	1	1	1	2	1	1	2	7·50	The filter was covered with a dense black slimy deposit
Brook before cleaning	1	1	.	.	1	1	1	.	1	.	1	1	1	8·00	Brook was shallow and full of vegetation
Pond	2	2	.	.	2	2	2	1	1	2	1	2	1	8·00	Diameters 10 and 15 yards approx.
Relative frequency of pathogens in Lea Valley waters	41	17	1	9	34	25	13	6	6	28	11	23	13		

of infection. The enumeration of the various members constituting the microscopic population of the water samples are necessarily incomplete, for whilst it is possible readily to distinguish some members, others such as *Phytophthora cryptogea* or *Colletotrichum oligochaetum* are identifiable in mixed culture only with difficulty. Owing to the uncertainty in determining the latter it has been omitted from the table. The identification of *Bacillus lathyri* (4), causative of "stripe" of tomatoes, is a lengthy process, but in six samples this was attempted and the bacillus found in three. With present technique, quantitative results do not seem possible and consequently in comparing the fungal populations of the various samples, the number of different species of parasites found has been taken as an indication of the purity of the water.

The results given in Table I clearly show that the extent of fungoid contamination of the waters varies with the source from which they are drawn. The purest waters are those from the Water Company (Metropolitan Water Board) and deep artesian wells; there being an average of 1·25 to 1·50 pathogens per sample. It is possible too that the fungi found in these waters may be nozzle contaminations rather than actual contaminations of the water supply. Wåter which had passed through an uncovered tank was more contaminated than that from a covered one. There is evidence that the former collects fungus spores and becomes a source of contamination to the water passing through. Samples from wells into which the surface drainage can readily pass yield a large number of species. Deep wells of this kind were only slightly less contaminated than shallow wells, but shallow wells placed some distance away from the glasshouses were generally purer than those surrounded by houses. Again, those wells, whose waters contained a large amount of decaying organic matter, were richer in fungi than those whose waters were clearer. Water from brooks and ponds was also highly contaminated, but a slight reduction in the number of fungus species resulted from thoroughly clearing away the vegetation. It will readily be seen that the Water Company's supply and water drawn from deep artesian wells are comparatively free from fungoid contaminations, while water from wells polluted by surface drainage, as well as brooks and ponds, may be a very serious source of infection. The various species of *Phytophthora* appeared in these latter sources.

Isolations.

Representative isolations of the various pathogens were made, grown in pure culture and tested for pathogenicity. To ascertain the

extent of the growth, which could be made by the various fungi in the actual well waters, samples of water were obtained from six different wells; the samples varying in the quantity of organic matter they contained. Tubes were filled at the rate of 10 c.c. per tube, plugged, sterilised and inoculated from the pure cultures of the fungi isolated from the water samples. In every case an appreciable growth resulted within 20 days and within 40 days the growth was sufficiently strong to show that the waters tested were moderately good media for the growth of the pathogens. There was a striking relation between the rate of growth and the amount of organic matter in the water; the rate of growth in the clearer waters being slower than that in the impure waters.

The purification of contaminated waters.

The purification of contaminated waters may be attempted along three main lines, namely filtration, sterilisation by heat or sterilisation by chemicals.

Filtration.

The beneficial results obtained by this method are patent when one considers the efficiency of the filter beds utilised in the preparation of pure water for domestic purposes. To be efficient, such filters require constant overhauling and must be of fairly large extent and thus the utilisation of filter beds on any but the largest nurseries does not come within the limits of practice. Certain means of roughly filtering contaminated waters have been attempted with beneficial results. In one case the contaminated water was allowed to percolate through a tightly packed sand and coke filter, some two feet in diameter and six feet in length. The resulting water proved to be free from fungus spores for the first three months during which the filter was in use. After 12 months' use the efficiency of the filter was slightly reduced, but there was still a great reduction in the number of fungi present in the water after filtration.

Sterilisation by chemicals.

In view of the desirability of obtaining results fairly rapidly, it was only possible to attempt a rough determination of the toxic action of various chemical compounds upon the vegetative and "summer" spore forms of fungi selected owing to their comparatively great commercial importance as disease producers. The type of fungus growth used in the tests was as follow:

Pieces of mycelium of *Phytophthora cryptogea*, in such quantities as are easily picked from a culture by an ordinary platinum needle, were

taken from luxuriantly growing cultures from three to eight weeks old, on Quaker Oat agar. With *Phytophthora parasitica*, small pieces of mycelium as before, but bearing numerous ripe sporangia, were placed in sterile water for an hour before the chemical was added, so that numerous zoospores were liberated. The strain of *Colletotrichum oligochaetum* used was isolated from lesions on cucumber leaves from a commercial nursery in 1920. On Quaker Oat agar acervuli are produced, yielding large quantities of spores, and a suspension of these was prepared by pouring 10 c.c. of sterile water into a tube containing a culture, gently shaking and pouring off into a sterile tube. For *Cladosporium fulvum* suspensions of spores were made in sterile water under conditions as aseptic as possible by scraping the spores from "mildew" spots on freshly gathered tomato leaves. Control plates gave comparatively pure growths of *Cladosporium fulvum*.

In carrying out the tests, the suspension of mycelium or spores was added in 1 c.c. lots to sterile test-tubes. The necessary amounts of sterile water and of solutions of the chemical were added to bring the final volume up to 2 c.c. at the desired strength. After shaking well the suspensions of spores and chemicals were allowed to stand for about 20 hours. Control tubes were set up in each case and invariably gave rise to good growths when plated. After standing for 20 hours the suspensions were transferred to sterile centrifuge tubes, diluted to 10 c.c. with sterile water, centrifuged, and after decanting the top 9 c.c. the remaining 1 c.c. containing mycelium or spores was plated—*Phytophthora* spp. on Quaker Oat agar and *Colletotrichum* and *Cladosporium* on Waksman's modified egg-albumen agar. The plates were incubated at temperatures approximating to the optimum for the particular fungus concerned, viz. *Phytophthora parasitica* at 29° C., *P. cryptogea* at 25° C., *Colletotrichum oligochaetum* at 25° C., and *Cladosporium fulvum* at 22° C.

Sterilisation by heat.

The suspension of mycelium or spores was placed in a sterile test-tube and heated for one minute in a water bath at different temperatures; the latter being recorded by means of a thermometer in a second test-tube in the water bath. After heating, the suspension was plated out and incubated as in the chemical tests.

The results in Table II show the limits within which the lethal concentration lies expressed as parts per 100,000 of chemical in the water suspension. The first number in the columns indicates the concentration at which the compound begins to have a toxic effect and the

Table II.

Table showing the limits within which the death-point lies expressed as parts per 100,000 *of chemical in the water suspension (all liquids were measured by volume and solids by weight).*

	Phytophthora parasitica Mycelium and conidia	Phytophthora cryptogea Mycelium	Colletotrichum oligochaetum Spores	Cladosporium fulvum Spores
Mercuric chloride	5–20	5–20	0·5–1	1–2
"Chloros*"	25–50	20–50	1–5	35–50
Formaldehyde	10–50	5–50	50–70	100–300
Formic acid	10–50	10–50	10–50	50–200
Potassium permanganate ...	40–100	40–100	5–350	10–500
Copper sulphate	10–50	10–40	10–40	40–100
Ammonium polysulphide solution, 1919	100–300	100–200	10–30	10–30
Phenol	300–500	300–500	70–200	70–200
Sulphuric acid	100–500	5–30	100–500	500–2000
Calcium bisulphite solution ...	300–1000	300–1000	100–300	100–300
Potassium sulphide	over 2000	1000–2000	10–50	50–200
Acetic acid	100–200	50–200	50–200	200–300
Ferric chloride	50–2000	50–200	600–2000	600–2000
Ferrous sulphate	over 2000	over 2000	over 2000	over 2000
Zinc chloride	„ 3000	„ 3000	„ 3000	„ 3000
Hexamine	„ 3000	„ 3000	„ 3000	„ 3000
Potassium sulphate	„ 3000	„ 3000	„ 3000	„ 3000
Potassium biphosphate ...	„ 5000	„ 5000	„ 5000	„ 5000
Sodium carbonate	„ 5000	1500–5000	„ 5000	„ 5000
Sodium formate	„ 5000	over 5000	„ 5000	„ 5000
Sodium nitrate	„ 10000	„ 10000	„ 10000	„ 10000
Temperature to which water must be raised to kill mycelium and "summer" spores	60° C.	50° C.	60° C.	70° C.

* Proprietary solution.

second number the concentration which is completely toxic. Thus five parts in 100,000 mercuric chloride retards the growth of *Phytophthora parasitica*, while 10 parts in 100,000 kills the spores and mycelium. Where "over 2000" appears in a column, it indicates that the number following "over" is the lowest concentration which shows any toxic effect. The final lethal concentration in these cases is too high for practical purposes and has not been determined. The compounds are arranged in order of relative toxicity; the most effective ones appearing at the top of the list. It must be understood that the concentrations given are toxic only to the vegetative and thin-walled spores of the fungi employed, and not to the thick-walled resting spores. Thus, while they may not completely rid the water of its infections, they practically do so by

killing the mycelium and "summer" spores which it contains. Only the thick-walled resting spores remain and these have not been found to any great extent in the water samples tested.

By raising the temperature of the water to 70° C., for one minute, the mycelial and "summer" spore forms of the fungi tested were killed. Complete sterilisation of the water is obtained by raising the temperature to boiling point for one minute.

Control.

Investigation of the cause and source of any plant disease is not complete unless the water supply has been carefully examined as a possible source of infection and in cases where it is probable that this is contaminated, steps must be taken to purify it.

The first process should be the thorough cleansing of the well, by removing as much algae and decaying plant material as possible, for a surface scum of the former assists the fungi mechanically and the latter makes the water a good medium for fungal growth. Copper sulphate used at the rate of 10 lb. to 1,000,000 gallons of water will keep down the growth of algae, which are so objectionable in wells, tanks, etc. When the water is very thick with particles of decaying plant material it may be cleared by precipitation with alum and sodium carbonate, added at the rate of 18 lb. potash alum and 5 lb. sodium carbonate for every 25,000 gallons of water. Perchloride of iron at the rate of 9 lb. per 25,000 gallons of water is also a useful clarifying agent. Of the chemical compounds tested, mercuric chloride and "chloros" are the most toxic to fungi. Mercuric chloride, however, is highly poisonous to man and it is not advisable to use it unless very special precautions are taken to prevent the workers drinking the treated water. No such restrictions apply to "chloros" in such a highly diluted condition. It should be added at the rate of 50 gallons to 100,000 of water, but half that concentration will give protection against the "summer" spore forms of *Phytophthora* spp. Complete sterilisation of the water is only obtained by boiling, but beneficial results have been obtained by arranging the pipes leading to the tank in such a manner that they are heated by passing through the boiler. While it may not be so necessary to supply the more mature plants with disease-free water, it is advisable to sterilise in some way all the water used in the early stages of propagation, when the plants so readily succumb to "damping-off" and "foot rot."

In choosing the source of water supply the following points should be considered. Stagnant brooks are really natural sewers and may be

badly contaminated with disease organisms. Ponds full of algae and weeds are suitable breeding places for fungi and bacteria. Shallow wells receive surface drainage from the neighbouring land and are liable to be contaminated, especially if heaps of decaying plants are deposited in their vicinity. Deep artesian wells are the purest and safest. Care should be taken to cover all wells and tanks to prevent contamination with debris that otherwise might gain entrance.

Summary.

1. Nursery waters in the Lea Valley have been examined and some shown to be an important source of disease.

2. Contaminated waters may be purified by filtering, boiling and by addition of certain chemicals.

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STUDIES IN BACTERIOSIS. V

FURTHER INVESTIGATION OF A SUGGESTED BACTERIOLYTIC ACTION IN *PROTEA CYNAROÏDES* AFFECTED WITH THE LEAF-SPOT DISEASE

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(With 1 Text-figure.)

In a previous paper[1] attention was drawn to the possible occurrence of a bacteriolytic process analogous to that found in animals. The existence of some such phenomenon was indicated by the marked localisation of the disease, by the difficulty experienced in the isolation of the parasite from the diseased areas, and by the appearance of the diseased tissue, the cells of which showed no well-defined micro-organisms. The presence of bacteria was suggested by the granular appearance of the contents of many of the cells, giving the impression of a bacterial zoogloea embedded in resinous material. The degree of granulation of this substance varied in different cells, and its affinity for bacterial stains varied in proportion to the degree of granulation. These observations seemed to suggest a process of dissolution of the bacterial zoogloea.

The present work was undertaken to elucidate this phenomenon and to determine the fate of the bacteria after they had invaded the leaf tissue and produced the *leaf-spot*.

Fresh isolations of the parasite, *Pseudomonas Proteamaculans* P. and S., were made and again the old difficulty was experienced: of twenty-two attempts, only five yielded cultures of the causal organism. It does not appear that any seasonal fluctuation could account for the numerous failures, since of the five successful attempts one was made in November, two in March, and two in July.

The method employed was as follows: The surface of the leaf was

[1] Paine, S. G. and Stansfield (1919). *Ann. App. Biol.* VI, p. 27.

washed rapidly with 65 per cent. alcohol, and a portion containing one of the diseased areas cut from the leaf and ground under aseptic conditions with sterile sand and 5 c.c. of sterile water. After a lapse of a minute or so for the settling of the sand, 1 c.c. of the liquid was further diluted with 4 c.c. of water, and 1 c.c. of this dilution was plated in bouillon agar. In the successful experiments the number of colonies of *P. Proteamaculans* was never great, and since these represented roughly one twenty-fifth of the total number in the leaf tissue taken, it must be concluded that the number of viable organisms present was much less than might have been expected considering the extent of the disorganisation of the tissue. The method may perhaps appear rather drastic and liable to loss of bacteria by adsorption on the sand and detritus and by their actual destruction in the grinding process. A certain amount of loss does occur, but as shown later (footnote, p. 23), it is not more than about 20 per cent. of the organisms present. It seems then that some factor militating against the bacteria comes into action after a lesion of a certain size has been produced.

In this case a large percentage of the spots might be expected to contain no viable organisms. To determine this percentage 100 "spots" were cut aseptically from leaves and dropped each into a separate tube containing 5 c.c. of bouillon; in order to aid the escape of bacteria each area of the diseased tissue was usually cut through the middle. After incubation for three days the broth in all cases remained perfectly clear and after streaking the broth from 75 tubes across the surface of nutrient agar plates, no growth of bacteria was obtained. The experiment was repeated with 64 diseased spots and again after three days none showed the presence of *P. Proteamaculans*, although 17 tubes showed turbidity by other organisms. Five of these examined after seven days' incubation still showed no development of *P. Proteamaculans*; two tubes, however, which had been put aside were found to contain this organism when examined ten weeks later. It seemed possible that the reddish-brown pigment which had diffused from the tissue and coloured the broth quite strongly in certain cases, or perhaps some other diffusible toxin from the leaf, had a retarding influence upon the rate of development of the organism. Six of the most strongly coloured tubes were therefore inoculated from a pure culture of *P. Proteamaculans*, and although growth was not very vigorous at first, all six developed the organism and were strongly turbid after a week or so. Marked retardation of growth was therefore not due to this cause. A third set of 65 tubes was prepared as before. At the end of nine days the broth in all cases was clear

and bright, but in 44 (and seven days later in 48) cases a colourless gelatinous substance was observed around the piece of tissue lying at the bottom of the tube. On staining, this appeared as a zoogloea of bacteria of similar shape to *P. Proteamaculans*, but smaller in size. This did not develop further and was interpreted as a swollen mass of the gum-like substance containing dead bacteria. This phenomenon of the swelling of the gum has been observed in the case of dead or dying leaves kept under moist conditions; small beads of the yellow gum were seen on the surface of the diseased spots, and these could be removed, spread on a slide and stained. They showed the same appearance of small embedded bacteria as described above. In order to test the viability of these organisms small masses of the gum of the size of a pin's head and containing some millions of the bacteria were ground with sterile sand and water, and the water then plated on agar and incubated. Four experiments of this kind were made; in three cases no growth was obtained, but in the fourth numerous colonies developed. The experiments differed in that the gum used in the three with negative result was so dry as to be quite brittle, while in the fourth it was noticeably much softer in consistency. The presence of living organisms in the gum exuded from a dead leaf would seem to discount the hypothesis of active bacteriolysis taking place within this medium.

The foregoing experiments emphasised the difficulty of isolating the parasite, but failed to determine the percentage of spots containing viable bacteria. The number of cases where *P. Proteamaculans* appeared was very few, but it was impossible to say how far the sterilisation of the leaf surface had contributed to this result. Experiments with unsterilised leaves were equally unsuccessful through the difficulty in recognising the parasite in the presence of the numerous other organisms which developed. One of these, a small *Sarcina*, very closely resembled diplo-forms of the *Pseudomonas*.

Experiments were next made to determine the fate of bacteria in spots produced by artificial infection. The surface of a fresh leaf of a young seedling of *Protea cynaroïdes* was first washed with 60 per cent. alcohol, scratched in several places with a sterile needle and the scratches covered with uniform drops of a bouillon culture of *P. Proteamaculans*. On the sixth day the leaf was washed with sterile water to remove organisms from the surface. The first spot was cut out on this day and the others at intervals of every second or third day, and the number of bacteria counted. The spots were cut out with a sterile cork-borer, ground in 5 c.c. of sterile water with 1 gm. of sterile sand, suitable dilution plates

were made in bouillon agar and the colonies counted after two days' incubation[1]. Fig. 1 shows the result of this experiment. It will be seen that the numbers rose from 1,242,000 on the sixth day to 2,808,000 on the fifteenth day, and then fell rapidly to 16,000 on the thirty-second day, when the experiment came to an end. This experiment was made in July. A repetition was made in October of the same year, drops of a suspension of the organism in sterile water being used instead of a bouillon culture. This series was very unsatisfactory in the number of successful inoculations; out of 24 spots only the following five records were

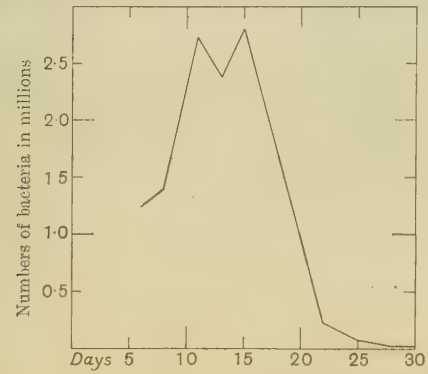

Fig. 1. Showing the rise and fall in the numbers of bacteria in leaf spots artificially infected with *Pseudomonas Proteamaculans*.

obtained: after 9 days, 1,280,000; 15 days, 1,576,000; 27 days, 1,808,000; 35 days, 1,336,000; 40 days, 485,000.

A sixth spot showed an exuded drop of mucilage which has been regarded as the first sign of active infection only on the forty-third day and gave a count of 1,850,000. A seventh spot developed actively some time later.

These experiments showed clearly that the rate of destruction of the organism was not very rapid, and again negatived the hypothesis of the production of a bacteriolysin by the invaded cells.

[1] The degree of accuracy of this method was tested in the following manner: First, as to the uniformity of the individual drops of bacterial suspension applied to the leaf—nine drops were plated separately and counts made after two days' incubation varied from 601 to 746. Second, as to the number of bacteria lost by adsorption on the sand and ground-up tissue—four experiments were made comparing the numbers in drops of suspension before and after grinding with sand and sterile leaf tissue: in one of these the loss was 20 per cent. and in the other three 9 per cent.

The question of auto-intoxication of the bacteria was next considered. A broth culture of the organism was placed out in uniform drops upon small squares of sterile mica and maintained in a moist Petri dish. Periodical counts of the organisms were made in bouillon agar after suitable dilution in sterile water. The numbers of living bacteria remained about 3–4 millions for five weeks (disregarding one low count of 1,760,000) and at the end of eight weeks, when the experiment was concluded, they still amounted to nearly one million. The falling off of the numbers in the infection experiments, which became marked in 30 days and 40 days in July and October respectively, was therefore not due to auto-intoxication of the invading organism.

The resistance of the organism to desiccation was next investigated. Two measured drops of a broth culture were placed on squares of sterile mica. One was immediately washed off with sterile water and plated. The number of organisms present was determined as 1,328,000. The other drop was allowed to dry for 24 hours and was then washed and rubbed off the mica with a sterile glass rod. The numbers of bacteria were found to have fallen to 11,000. In a second experiment the numbers were reduced from 890,000 to 15,000 in 24 hours, to 6400 in four days and to zero in eight days.

In order to approach more nearly the conditions of nature the experiment was repeated using in the place of the mica small pieces of *Protea* leaf which had been dried in air after having been heated in the autoclave. A water suspension containing some four million organisms per drop was employed and the result was as follows: after 1 day, 2,044,340; 2 days, 569,050; 3 days, 7069; 6 days, 7214; 9 days, 802; 13 days, 320.

Obviously desiccation is an important factor and no doubt accounts for the death of the organism in the diseased tissue. The non-viability in the dry gum and viability in moist gum as described on p. 22, is explained in this way, as also is the relatively slower onset of sterility in the October infection experiment as compared with the July experiment described above, drying presumably being more rapid in July.

Under favourable conditions the moisture content of the invaded tissue remains sufficiently high to maintain the life of the organism for some considerable time. The organism has been re-isolated from artificially infected spots 65 and 75 days after infection. Even after the lapse of 16 weeks in one case two colonies of *P. Proteamaculans* were obtained on grinding the diseased spot and plating.

The rapid growth of the organism and the area of the spots produced

are evidently restricted by cork formation and production of wound gum, and the ultimate death of the organism is the result of desiccation within the area enclosed by the cork.

MICROSCOPICAL EXAMINATION OF THE TISSUES.

The fact that the gummy material present in the spots is softened and slowly dissolved by chromic acid has rendered it possible to get fair microtome sections varying from 4–6μ in thickness after the use of chromic acid fixatives. The most satisfactory stain for the bacteria in the tissues was found to be carbol fuchsin followed by light-green in clove oil.

Sections through an artificially produced spot seven days after infection show masses of a small diplo-bacillus with thick capsule lining the cavity produced by the infecting needle and extending into the neighbouring cells and intercellular spaces. The shrivelled cells surrounding the cavity contain clear yellow gum, those beyond a paler vacuolate gum, mostly crammed with bacteria, but in certain cases quite clear and structureless. The view that the granular appearance of the gum in many cells is due to the inclusion of bacteria is confirmed by these sections, and also by the fact that the gum exuded by the dead leaves contains the diplo-bacillus, but in a smaller form than that obtained from an agar culture. The strong affinity of this granular gum for bacterial stains such as carbol fuchsin is thus accounted for.

The infected spots are sometimes, but not always, cut off by a definite layer of cork: multiplication of the host cells has usually occurred and their walls do not give the cellulose reaction with iodine and sulphuric acid: they and the gum in the intercellular spaces stain red with phloroglucin, possibly owing to the presence of wound gum.

The clear structureless gum which accumulates in the cells bordering the spot, mainly in the palisade parenchyma, before the plastids and cell contents are disorganised, is coloured rose-red in fresh hand sections, and on treatment with normal caustic soda turns a bright chlorophyll green. In microtome sections where it is pale and colourless it stains green with chlorophyll extract and yellow with potash, which seems to indicate that it contains something allied to suberin.

These observations show that the host plant has considerable powers of resisting the invasion of the parasite by producing cork, the strict localisation of the disease being thereby accounted for.

Summary.

1. The production by the cells of *Protea cynaroïdes* of a bacteriolysin against the organism which produces a leaf-spot disease, suggested in a former paper, has not been confirmed.

2. The bacteria enter the leaf by way of the stomata; the local lesions produced are prevented from spreading by the development of wound cork.

3. The causal organism succumbs very readily to the desiccation to which it is subjected owing to the cells which it attacks being cut off by the wound cork from their water supply.

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THE KILLING OF BOTRYTIS SPORES BY PHENOL[1]

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(With 11 Text-figures.)

Several workers have studied the manner in which bacteria are killed when submitted to the action of poisons: their conclusions are referred to later. One might expect that in the case of fungi the findings would be the same, but little accurate and detailed information is available, and as there is still a great deal of uncertainty, partly as to the facts, and still more as to their interpretation, in the killing of bacteria, it seemed possible some further light might be obtained by a study of the problem, using fungi as the test object.

The organism selected was *Botrytis cinerea*, the strain used being the one described by Brierley (1). From a single spore of this strain cultures were obtained on Czapek's medium, and in all the experiments detailed here Czapek's has been the only medium used.

Method.

The method adopted was as follows. The spores were sown on Czapek-agar slopes, which were kept at room temperature in a subdued light till used. From these a suspension of spores was prepared of the desired density in distilled water. In removing the spores care was taken not to touch the subjacent medium and to obtain a suspension as free as possible from hyphae. This was done by laying lightly on the tops of the conidiophores a platinum loop filled with distilled water: the spores adhere to the film of water on the loop and are transferred to a tube of water and washed off. By repeated application of the loop a suspension is obtained of practically pure spores. To a measured volume of the suspension, previously brought to the desired temperature, was added an equal volume (also previously warmed) of the toxic agent to be examined, in such strength that the final mixture was of the desired concentration. The mixture was maintained in a water-bath at

[1] A grant in aid of publication has been received for this communication.

the temperature selected in each experiment, and kept stirred, and samples were withdrawn at intervals for examination. The volume of the mixture was not less than 10 c.c., usually about 20 c.c., and the samples were of 0·1 c.c. volume, except when the suspension was very dense, in which case smaller samples were taken. Each sample was immediately dropped into a centrifuge-tube containing 5 c.c. of distilled water (in experiments with high concentrations of phenol 10 c.c. were used), and rapidly centrifugalised. The supernatant fluid was removed till about 0·25 c.c. remained, and to this the deposited spores were stirred up to form a suspension. From the latter a standard platinum loopful was transferred to a coverslip, on which had been placed a standard loopful of Czapek's fluid, and the two drops well mixed. The coverslip was then placed on a wet Dieghton cell, and this incubated at 20° C. for 22 to 24 hours. Two or four such cells were prepared from each sample. After incubation they were examined with a Zeiss C objective and No. 6 eyepiece, and the proportion of spores that had germinated ascertained by counting successive fields. This is readily done, the spores capable of germination protruding in the case allowed hyphae or germination tubes which are easily seen. As a rule, the whole drop was counted, and to get reliable figures it is necessary to count several hundred spores from each sample. Four or five hundred spores are enough, but in most cases six or eight hundred, and sometimes more, were counted. It is sometimes stated that more spores germinate round the edges of the drop than in the centre (1). I was unable to reproduce this result under the favourable germination conditions used in these experiments, but it is advisable to count all spores in the whole drop, in order to avoid any error from this source.

The method gives uniform and satisfactory results. The first experiment given below is set out in detail to illustrate the amount of variation obtained in duplicate slides. The variation does not exceed the unavoided error of sampling. But to get consistency the technique must be carried out with rigid uniformity and scrupulous attention to details. All slides, pipettes, coverslips, etc. are, of course, plotted and sterilised. There would seem to be great opportunity for contamination in mixing the drops on the slips, but as a matter of experience contamination is a rare occurrence—I estimate it at less than 2 per cent. of all preparations made. The bottom of the wet Dieghton cell in which the rings were mixed with beeswax was covered with Czapek solution, the coverslip laid on without vaseline or other cement, and the whole cell placed in a covered glass dish containing distilled water to prevent drying. In

cases where it was impossible to complete the counts quickly enough, a few drops of chloroform were added to the cell after incubation. This arrested all further growth and the slides could be counted at leisure. Some device of this sort was necessary, because otherwise the hyphal growth becomes so profuse as to make accurate counting impossible.

The dilution of the sample in the centrifuge tube arrests the action of the phenol by lowering its concentration to a point much below that which has any perceptible effect on the spores in the time given. The number of spores contained in the final mixtures was determined by direct counting in the Thoma-Zeiss haemocytometer.

In Table I is given the result of an experiment with 0·4 per cent. phenol and spores taken from the tube 27 days after inoculation. During the first 20 minutes apparently none of the spores are killed, but in the second 20 minutes about 1·5 per cent. die, and in the third 20 minutes a further 7·5 per cent., in each successive interval more and more dying

Table I. Expt. of 23. 10. 19. Spores 27 days old; 150,000 per 1 c.c. Phenol 0·4 per cent. Temp. 25°.

Sample taken in minutes	Slide No.	Number counted	Per cent. germinated	Total number	Per cent. germinated	Proportion
0	1	353	91·5	590	91·5	100
	2	237	91·5			
30	1	300	91·0	651	92·0	100·5
	2	351	92·8			
50	1	353	87·2	579	85·3	92·9
	2	226	82·5			
70	1	541	79·1	973	76·0	82·8
	2	432	72·2			
80	1	142	61·2	573	60·5	65·9
	2	431	60·3			
90	1	453	48·9	887	50·8	55·3
	2	434	52·7			
100	1	359	35·3	767	35·2	38·3
	2	408	35·0			
110	1	601	26·4	1011	24·2	26·3
	2	410	20·9			
120	1	262	24·4	585	22·3	24·2
	2	323	20·7			
130	1	446	19·2	814	15·4	16·7
	2	368	10·8			
140	1	292	6·16	655	6·71	7·31
	2	363	7·27			
150	1	350	3·7	484	4·95	5·39
	2	134	8·2			
160	1	442	2·03	808	2·84	3·09
	2	366	3·82			
170	1	540	1·85	827	1·69	1·84
	2	287	1·39			

until the period of 80–100 minutes is reached. During this interval nearly 30 per cent. of the total number of spores are killed. Thereafter fewer and fewer are killed in each time-interval, until in the 160–180 minutes interval only about 1 per cent. die; and the last survivor would not succumb until some four hours or more had elapsed from the beginning of the experiment. The whole curve, which may be called a survivor curve, has a sigmoid shape (see Fig. 1).

In Tables II, III and IV are given experiments with higher amounts of phenol, viz. 0·5, 0·6 and 0·7 per cent., and three of these are also plotted in Fig. 1. The close scale of the figure somewhat obscures the character of the curves with the higher strengths, but if plotted on a more open scale they will be seen to conform to the general type of the 0·4 and 0·5 per cent. curves.

Table II. 18. 9. 19. 27 day spores. 200,000. 25·2°. Phenol 0·5 per cent. Control: 89·5 per cent. of 777 spores germinated.

Sample taken in mins.	Number counted	Per cent. germinated
0	777	100
20	683	94·6
40	735	73·1
60	913	28·9
80	1135	8·37
100	1066	2·3
120	744	1·9
140	1465	0·76
160	1248	0·71

Table III. 30. 10. 19. Phenol 0·6 per cent. 34 day spores. 280,000. Control: 83·88 per cent. germinated in 515. 24·8°.

Sample taken in mins.	Number counted	Per cent. germinated
0	515	100
3	289	95·3
6	541	93·3
9	757	76·6
12	456	66·6
15	357	45·3
18	885	16·8
21	694	13·8
24	637	2·6
27	835	0·84
30	1514	0·39

After 39 mins. two spores only germinated; after 42, 45 and 48 mins. none; after 51 mins. one; and after 54 mins. none.

Table IV. 1. 10. 19. Phenol 0·7 per cent. 18 day spores. 25°. Control: 86·95 per cent. of 914 spores germinated.

Sample taken in mins.	Number counted	Per cent. germinated
0	914	100
1.5	1503	71·3
2.5	632	52·1
3.5	1143	36·0
4.8	524	19·2
8.0	1195	2·04
11.25	1125	1·57

These experiments have been frequently repeated and always with the same general result. In experiments performed on different days and with different spore cultures the minor details never completely correspond, and it is almost impossible at a later date to reproduce exactly the result obtained on a previous occasion. For this many factors are responsible. The age of the culture and the number of spores used are of great importance, as will appear later, but the chief source of the discrepancies lies in the almost insuperable difficulty of obtaining on different days suspensions of spores which are perfectly alike. Small differences in the degree of moisture in the culture tubes, the tightness or looseness of the cotton wool plugs affecting the oxygen supply, and

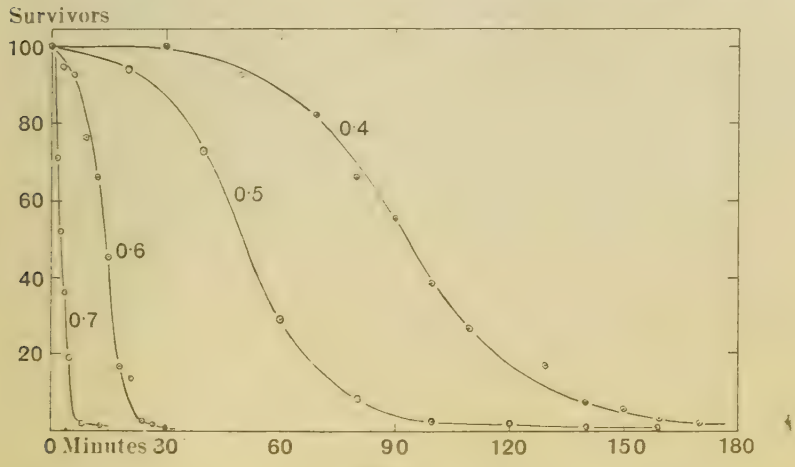

Fig. 1.

the like, modify the rate and character of the development of the spores, and this is reflected in the mortality rate. But the general character of the curves remains the same, a sigmoid curve with a stage of increasing steepness, a maximum, and finally a stage of decreasing steepness, flattening out more and more as time goes on.

This is unlike the curve generally accepted as the typical mortality curve for bacteria exposed to disinfectant agents, such as phenol, corrosive sublimate, heat, sunlight, etc. Since the papers of Chick in England (3, 4) and Madsen and Nyman in Denmark (5), the typical mortality curve has been recognised as a logarithmic one, corresponding in shape to the unimolecular curve, in which the number dying at any

moment is strictly proportional to the number alive at that moment. These workers do not as a matter of fact always obtain curves of this type—many of their results indeed depart very widely from it. But the logarithmic type is held to be the true one, and the departures from it to be variations caused by factors independent of the disinfection process itself. The sigmoid shape of curve is, on the other hand, a type occurring with great frequency in biological investigations of this kind. The literature of haemolysis is full of examples of it. Even with bacteria some workers find curves of this type occurring with heat or chemical poisons, and some of the experiments with staphylococci recorded by Chick would seem to fit a sigmoid curve as reasonably as a logarithmic one. It occurs also with more highly developed organisms, *e.g.* it was obtained by Boycott in the killing of tadpoles by hot water[9]. A good example may be seen in a recently published paper on the habits of the Tomato Moth[10], where the poison used was a lead arsenate paste. The plants were sprayed with a suspension of the paste, and then infected with the larvae of the tomato moth. On successive days thereafter the survivors were counted, and the results are tabulated. It is found that the larvae die off gradually, some being dead the day after the spraying, others surviving a week or more. The explanation offered is that the larvae find the paste distasteful, and the majority cease feeding before they have taken a fatal dose. After a time hunger forces them to recommence feeding, until finally a fatal dose is absorbed by all. The death-rate, however, is probably an example of the same phenomenon as that described above. I have plotted the figures given by Lloyd and reproduce in curve form two of his experiments (see Fig. 2). The sigmoid curve is well illustrated and presents the same general features as the curves obtained with Botrytis spores.

It is not difficult to find an explanation of the peculiar shape of the sigmoid curve. We do not know what is the nature of the reaction occurring between the phenol and the spores, whether, for example, it is a chemical combination of the phenol with the protoplasm of the spore, or a physical partition of the phenol between the two solvents protoplasm and water, resulting in the coagulation and death of the spore (cf. Cooper[8], p. 1). We are equally ignorant of the nature of the resistance offered by the spores to the poison. It is, however, reasonable to assume that in a large number of organisms, such as those in the suspensions of Botrytis spores, the individuals will differ from one another in resistance, and that if we graduate the resistances and determine the number of individuals in each grade (construct, in fact, a

frequency table), and then plot the results graphically, the curve so obtained will conform more or less closely to the normal curve. This is in accordance with a large mass of biological experience, both anatomical and physiological; and is likely to be true, whatever may be the nature of the resistance offered—whether, *e.g.* it has a structural basis, such as thickness or composition of the spore coat, or depends on some such property as a capacity to neutralise or destroy the phenol as it enters the spore. If this be granted, then the sigmoid survivor curve is what we should expect to find. For in the mass of spores exposed to the phenol those will die first which have the lowest grade of resistance: and these are few in number. In each successive time-interval further grades of resistance will be overcome, and these grades

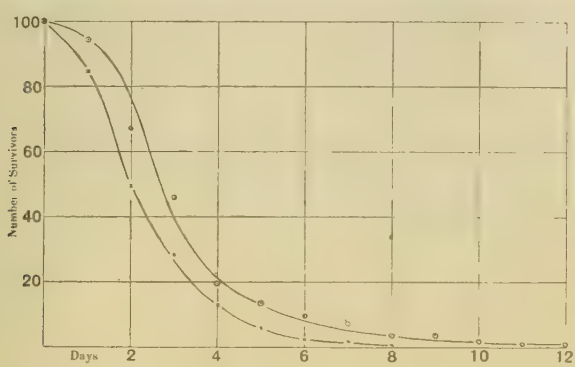

Fig. 2. Tomato moth larvae and lead arsenate spray (Lloyd).
⊙—⊙ 3 lbs. per gallon. ×—× 6 lbs. per gallon.

contain more and more individuals, so that in the successive intervals more and more spores will die: until the middle grade of resistance is passed. After that, in successive intervals more and more grades of resistance will be overcome, but as the individuals in these grades are less and less in number, fewer and fewer spores will die, until eventually only a very few individuals are left in each remaining grade. A curve expressing the numbers left alive after each time-interval, *i.e.* a survivor curve, will have a symmetrical sigmoid shape, and will resemble the survivor curve we obtain with 0·4 per cent. phenol, shown in Fig. 1.

The idea of explaining the different life-times of the spores by variations in the resistance of the individual spores was put forward at least as long ago as 1889 (16), and if we assume the variations to conform

to the normal curve, it gives an adequate and satisfactory explanation of the sigmoid curve.

If, however, we examine more closely the curves obtained with 0·5, 0·6 and 0·7 per cent. phenol, it becomes apparent that their shape, though still sigmoid in character, differs from that of the 0·4 per cent. curve, the difference being progressively more marked as the strength of phenol is increased. All the experiments recorded in Tables I—IV were done with adult spores in approximately equal numbers and at nearly the same temperatures. They may fairly be compared; and in the following table (V) the results of these and other[1] similar experiments done with each strength of phenol are brought together. The curves were drawn for each experiment, and the times required to kill 25, 50 and 75 per cent. of the spores read off, and from these readings the mean times for each strength of phenol obtained.

Table V. Times in minutes taken to kill 25, 50 and 75 per cent. spores by 0·4, 0·5, 0·6 and 0·7 per cent. phenol. In brackets are given the figures for each with the 0·7 per cent. times taken as unity.

Phenol per cent.	25 per cent.	50 per cent.	75 per cent.
0·4	90·25 (34·8)	103 (24·5)	118·5 (18·9)
0·5	34·50 (13·2)	45 (10·7)	53·5 (8·5)
0·6	9·12 (3·5)	11·23 (2·6)	13·48 (2·1)
0·7	2·6 (1·0)	4·2 (1·0)	6·25 (1·0)

It will be seen that the times required fall rapidly as the strength of the phenol is increased—*e.g.* while 90 minutes are required with 0·4 per cent. to kill 25 of each 100 spores, with 0·7 per cent. only 2·6 minutes are necessary. But it is also apparent that the ratios of the times required vary greatly, if we compare the 25 per cent. with the 50 and 75 per cent. columns. It takes 34 times as long for 0·4 per cent. phenol to kill 25 per cent. of the spores as for 0·7 per cent. to kill the same number: but it takes only 19 times as long for 0·4 per cent. to kill 75 per cent. as for 0·7 per cent. With the strong poison the time of killing is much more reduced in the early part of the process than it is in the later stages. The effect of increasing the quantity of phenol is to accelerate the killing much more markedly in the early part of the curve—*i.e.* increasing the phenol tends to remove the slow stage at the beginning of the curve and to reduce the sigmoid character: the curve tends more and more to approach the logarithmic type as the phenol is more and more raised.

[1] The cost of printing at present prevents these being given in full.

If we write the last table in a different form, taking as 1 the time required to kill 75 per cent. of the spores, we get Table VI.

Table VI.

Per cent.	25 per cent.	50 per cent.	75 per cent.
0·4	0·76	0·87	1·0
0·5	0·64	0·84	1·0
0·6	0·67	0·83	1·0
0·7	0·41	0·67	1·0
Unimolec. curve	0·20	0·50	1·0

The corresponding time-ratios for a unimolecular curve are added to this table, and it will be seen that the observed figures approximate more and more closely to the logarithmic type, the greater the strength of phenol used.

It is unfortunately not practicable to use higher concentrations of phenol at this temperature, 25° C. With 0·8 and 1·0 per cent. the reaction runs so fast in the early stages that it becomes impossible accurately to sample quickly enough. In 0·8 per cent. phenol, for example, 43·27 per cent. survive for 30 seconds, 5·7 per cent. for 1 minute, 3·6 per cent. for 1·5 minutes, and 0·39 per cent. for 2·5 minutes. Already in half a minute the number of survivors falls from 100 to 43, and it is impossible to observe the important early part of the curve.

Effect of Low Temperatures. An attempt was therefore made to work at much lower temperatures, in the hope of so slowing the reaction as to make observation practicable. All apparatus, the phenol solution, the suspension of spores, etc., were previously cooled to the temperature of melting ice, from 1·5° to 2·0° C., and the samples dropped into ice-cold water and centrifuged as quickly as possible. Control experiments showed that germination was not altered in numbers by the exposure to cold, but was rendered a little slower during the subsequent incubation. With 2·0 per cent. phenol at 2·0° all spores were killed in 20 seconds. With 1·5 per cent. phenol at the same temperature 1·32 per cent. survived 21 seconds, and 0·52 per cent. survived 41 seconds.

At the low temperatures it was possible to observe fairly satisfactorily the course of the reaction with 1 per cent. phenol. With higher concentrations the process was too rapid for detailed examination (see Tables VII–XI). It is apparent, however, that with 1·25 per cent. the sigmoid character is still present: and with 1 per cent., whether at 1·5° or 7–8°, the curve is frankly sigmoid (*v.* Fig. 3), as markedly as with 0·7 per cent. at 25°, even although the reaction proceeds more quickly. These results will be referred to later (*v.* p. 49).

Table VII. 24. 8. 20. 2° C. Phenol 1·31 per cent. 25 day spores. 290,000. Control: 94·15 per cent. germinated.

Time in seconds	Number counted	Per cent. germinated
0	855	100
20	927	8·69
40	1144	0·46
62	1217	0·43
120	533	0·39

Table VIII. 20. 8. 20. 2° C. Phenol 1·25 per cent. 21 day spores. 175,000. Control: 92 per cent. germinated.

Time in seconds	Number counted	Per cent. germinated
0	882	100
20	1117	47·8
40	513	1·9
60	687	0·31
92	1476	0·44

Table IX. 17. 8. 20. 1·5° C. Phenol 1·0 per cent. 18 day spores. 175,000. Control: 81·7 per cent. germinated.

Time in seconds	Number counted	Per cent. germinated
0	1422	100
21	988	68·4
41	619	94·5
61	508	90·2
90	400	82·6
123	464	53·5
154	488	27·8
180	524	17·6
212	499	5·14
250	710	2·92
300	934	1·30

Table X. 5. 8. 20. Phenol 1·0 per cent. 7–8° C. 37 day spores. 500,000. Control: 97·45 per cent. germinated.

Time in seconds	Number counted	Per cent. germinated
0	1334	100
0·5	1192	62·75
1·25	1794	10·98
2	1756	3·86
3·5	1690	1·33
4·5	1953	0·77

Table XI. 5. 4. 20. 12° C. Phenol 0·8 per cent. 23 day spores. 200,000. Control: 88·729 per cent. germinated.

Time in minutes	Number counted	Per cent. germinated
0	1568	100
1	860	93·96
2·25	622	94·39
4	828	88·08
6	576	67·89
8	1760	52·37
10	1715	26·74
13	1137	15·55
16	1208	5·12
19	1563	2·59
24	1399	1·69

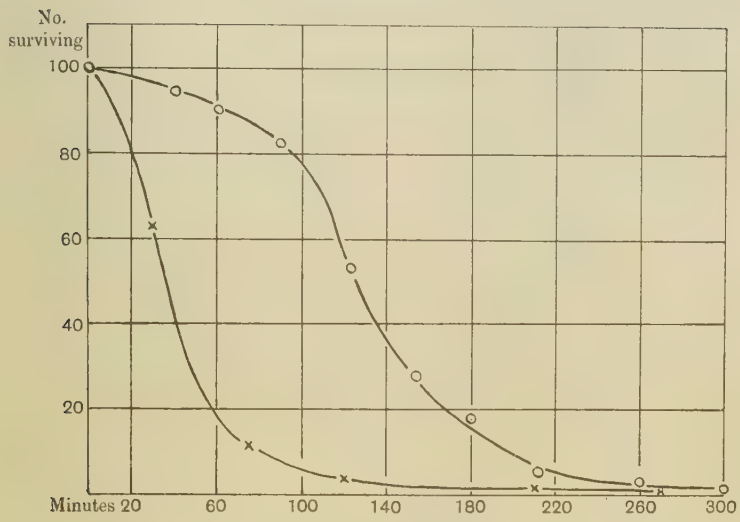

Fig. 3. Phenol 1 per cent.
○——○ Temp. 1·5° C. ×——× Temp. 7–8° C.

Effect of Varying Number of Spores. In Tables XII–XV are given a series of experiments, in each pair of which one and the same strength of phenol was used with suspensions of different density. Each pair was done at the same time with spores from the same suspension suitably diluted: and the results are strictly comparable with one another.

Table XII. 15. 12. 19. Phenol 0·4 per cent. 58 day spores. 25·2°.

Time in minutes	1,000,000 spores in 1 c.c.	11,400 spores in 1 c.c.
0	100	100
10	—	96·5
30	96·4	92·0
50	—	92·1
60	97·1	86·1
70	—	79·4
80	91·4	64·3
90	86·8	62·7
100·5	83·5	—
132·5	—	15·3
140	71·0	—

Table XIII. 8. 12. 19. Phenol 0·4 per cent. 51 day spores. 25°.

Time in minutes	2,700,000 spores in 1 c.c.	5000 spores in 1 c.c.
0	99·6	100
10	100·1	90·4
20	100·7	82·8
30	100·1	—
40	100·3	57·1
50	98·9	—
61	100	—
	Mean 100·0	

Table XIV. 26. 11. 19. Phenol 0·6 per cent. 50 day spores. 25°.

Time in minutes	400,000 spores in 1 c.c.	50,000 spores in 1 c.c.
0	100	100
5	90·1	43·7
10	27·0	16·3
15	9·32	4·50
20	2·08	—
25	0·99	0·96
30·5	0·31	—
35	0·185	—

Table XV. 17. 12. 19. Phenol 0·7 per cent. 50 day spores. 25·2°.

Time in minutes	3,000,000 spores in 1 c.c.	7000 spores in 1 c.c.
0	100	100
1	99·8	98·5
2	94·2	79·3
3	94·4	66·1
4	88·7	61·1
5	84·9	28·4
6	82·9	12·0
7	—	6·5
8	49·8	—
8·5	—	2·6
10	—	1·1
12	11·9	—
14	—	0·56

Table XVI. 2. 12. 19. Phenol 0·7 per cent. 45 day spores. 25·0°.

Time in minutes	1,200,000 spores in 1 c.c.	Time in minutes	32,000 spores in 1 c.c.
0	100	0	100
1·25	95·4	1·25	98·2
2·5	86·5	2·25	71·5
3·5	57·8	3·25	54·0
4·5	39·5	4·25	34·02
5·5	19·1	5·25	7·27
6·5	15·8	6·25	4·60
9·5	1·71	9·25	0·58

In Fig. 4 are charted the results of four experiments with 0·4 per cent. phenol and spores which varied in numbers from 5000 to 2,700,000 in each c.c. of the killing mixture. If we plotted on the same figure the curve already shown in Fig. 1, where 150,000 spores were used, its graph would come between the 11,400 and the 1,000,000 curves. It is evident that the increase in the number of spores has greatly slowed the rate of killing. With the largest number all are still alive after one hour. If this experiment had been continued I do not doubt that the curve would have eventually begun to drop like the others. The same thing is seen in the 0·6 and 0·7 per cent. experiments. The larger the number of spores employed, the more marked is the delay in the early part of the curve.

The bearing of these facts on infection is evident. It is well known that the larger the size of the inoculum the greater is the chance of a

successful inoculation being obtained, and that even fully virulent organisms may fail to produce infection when given in a small dose.

It is perhaps rather surprising that the change in the number of spores present should make so much difference in the times required to kill. The average dimensions of the spores used are about 10 μ long by about 8 μ broad. Each spore certainly weighs less than a piece of flint of the same size would weigh. But if we assume that the spore is a sphere 10 μ in diameter and that each spore has a weight of the same

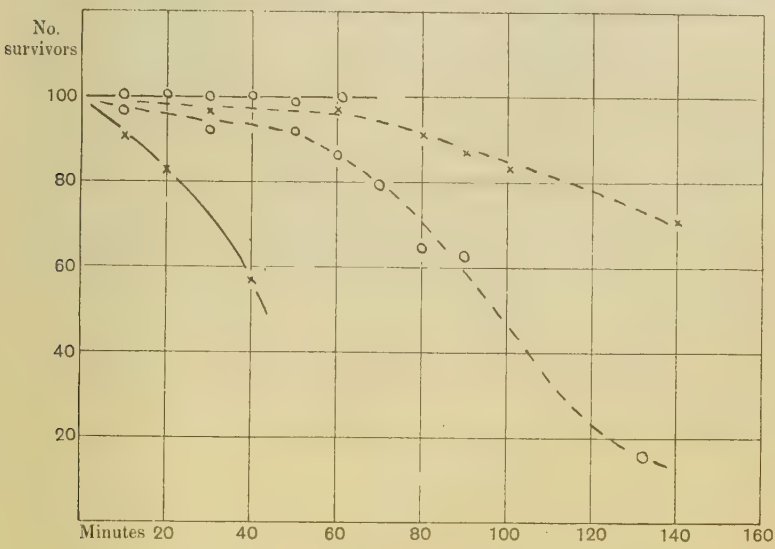

Fig. 4. Phenol 0·4 per cent.

—O—O 2,700,000 spores in 1 c.c. – – × – – × 1,000,000 spores in 1 c.c.
– – O – – O 11,400 spores in 1 c.c. —×—× 5000 spores in 1 c.c.

order as flint, viz. three times that of water—and this is greatly to exaggerate the weight of the spores—we have in a 0·4 per cent. phenol mixture, containing 1,000,000 spores in each 1 c.c., two and a half times as much weight of phenol as of spores. In a 0·7 per cent. mixture with the same number of spores there is present four times as much weight of phenol as of spores. We cannot suppose that the individual spore will take up nearly its own weight of phenol. I have not yet succeeded in estimating directly the amount of phenol actually removed from such solutions by the spores, for the quantities concerned are very minute,

and the disturbing influences difficult to eliminate. It seems probable, however, that the individual spore does not remove one-hundredth part of its own weight of phenol. If this is so, one might expect that a solution containing by weight several hundred times the amount of phenol taken by the spores would contain a practically infinite amount of phenol relatively to the spores, and that any alteration of the phenol-spore ratio would have a scarcely appreciable effect on the time of killing. But this, as we have seen, is not in fact the case.

Experiments with Young Spores. We have seen that for technical reasons it was not possible to obtain a logarithmic survivor curve when using adult spores, and that the use of low temperatures did not help. It is, however, possible to approach the question in another way by using spores of a different character. At room temperature on Czapek-agar Botrytis spores are first clearly visible to the naked eye on the 5th to the 6th day after inoculation of the tube. When first formed they are in the mass of a pale grey colour instead of the dark mouse colour they assume later. They are at this period already "ripe," since they are fully formed and germinate freely, but they are less resistant, as may be seen on a comparison of the times required for killing in the following tables with those already given. I accordingly carried out a series of experiments with young spores removed from the tubes soon after they were clearly formed. These are detailed in Tables XVII–XIX, and the graphs drawn in Figs. 5 and 6.

Table XVII. 22. 3. 20. 9 day spores. Phenol 0·7 per cent. Both series from same suspension. 26·3°.

Time in minutes	30,000 in 1 c.c.	240,000 in 1 c.c.
0	100	100
0·5	89·46	97·21
1	82·06	79·56
2	76·23	71·60
3	60·87	47·17
4	44·28	28·80
5	30·60	10·86
6	14·23	7·30
8	3·37	1·27
10	0·79	0·52
12	0·66	—
23	0·28	0·15

Table XVIII. 1. 4. 20. 7 day spores. Phenol 0·7 per cent. 26·2°.

Time in minutes	40,000 in 1 c.c.	Value of k
0	100	—
0·5	82·51	0·167
1	64·08	0·193
2	52·94	0·138
3	35·61	0·149
5	13·04	0·176
6	8·93	0·175
7	1·85	0·247
8	1·15	0·242
9	0·19	0·302
11	0·0	—

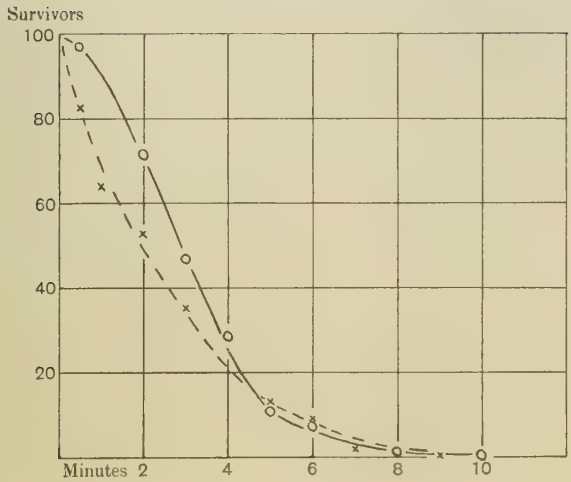

Fig. 5. Phenol 0·7 per cent.
O—O 9 day spores. × – – × 7 day spores.

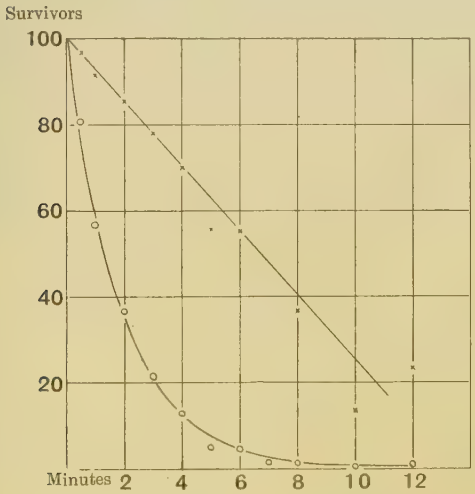

Fig. 6. Phenol 0·7 per cent. and 6 day spores.
The curve is drawn to the formula with $k = 0{\cdot}226$; the circles are the observed points. The crosses represent the logarithms (to base 10) of the observed number of survivors plotted against time.

Table XIX. 12. 4. 20. 6 day spores. Phenol 0·7 per cent. 25·2°.

Time in minutes	60,000 in 1 c.c.	Value of k
0	100	—
0·5	81·09	0·182
1	56·67	0·246
2	36·73	0·217
3	21·50	0·222
4	12·62	0·224
5	4·66	0·262
6	4·47	0·228
7	1·32	0·268
8	1·18	0·241
10	0·25	0·260
12	0·55	0·188
		Mean 0·230

With 9 day spores the sigmoid character is clear, more marked with 240,000 than with 30,000 spores to the 1 c.c. With 7 day spores, however, the character is altered. There is no longer perceptible to the eye an initial period of increasing rate and the curve falls steeply from the start and then slows off as before. It approaches much more closely to the logarithmic type than any of the preceding curves (*v.* Fig. 5). In the table (XVIII) is added the values of k on the assumption that the process follows the unimolecular formula $k = 1/t \log a/a - x$, where a is the original number of germinable spores and x the number dead at time t. The constancy of k is not so good as one would hope for, even in this type of work, and rises definitely towards the end. With 6 day spores, however (the youngest spores which it was found practicable to use), the value of k is as constant as one can reasonably expect, at least until only about 4 per cent. of the spores are still surviving (*v.* Table XIX). In Fig. 6 the curve is drawn from the formula with $k = 0{\cdot}226$, and the observed points fall very closely on the curve. In Fig. 6 also the logarithms of the observed values are plotted against time, and the points from 100 per cent. to under 10 per cent. fall very fairly on a straight line.

With sufficiently young spores, then, the curve is of the logarithmic type, and the sigmoid character has disappeared. It was of interest to determine whether with a low strength of phenol it would be possible to restore the sigmoid shape, when using spores which gave the logarithmic type with the higher strength. An experiment was accordingly performed with 6 day spores and two strengths of phenol. The same suspension was used in both cases, one-half being exposed to 0·7 phenol.

and the other half to 0·4 per cent. phenol, and the experiments were carried out simultaneously. The result is seen in Table XX and Fig. 7. The 0·7 per cent. curve shows no sigmoid character and approaches closely the unimolecular type, although in this particular experiment not strictly logarithmic (*v.* values of k in the table). The 0·4 per cent. curve, on the other hand, is frankly sigmoid. The same spores, then, give a sigmoid or a logarithmic curve according to the strength of phenol employed. The shape of the curve does not depend simply on the character of the spores used, but chiefly on the strength of phenol.

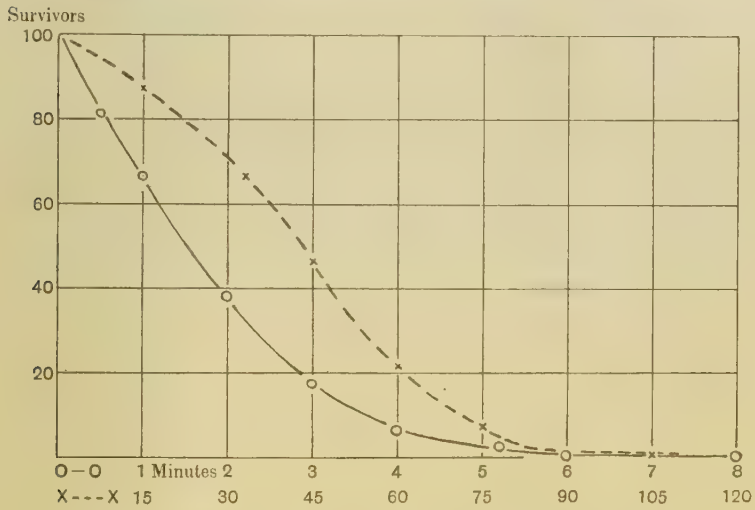

Fig. 7. Same suspension with × --- × 0·4 per cent. phenol and
○ —— ○ 0·7 per cent. phenol.
The 0·7 per cent. graph is plotted on a 15 times more open scale than the 0·4 per cent.

What, then, is the explanation of the logarithmic type of curve? It is evident that the explanation given for the sigmoid 0·4 per cent. curve will not serve here, at least not without modification. The 0·4 per cent. curve, when transposed into a frequency curve in the manner described, gives an approximation to a normal curve (*v.* Fig. 8), and the explanation derives its convincing cogency from the fact that the derived frequency curve approaches the normal curve so closely. In Fig. 8 is also drawn a strictly normal curve ($\log y/16 = -x/3113$). A better fit could no doubt be obtained by suitable adjustment, but it

Table XX. 20. 4. 20. 6 day spores. Phenol 0·4 and 0·7 per cent. with same suspension. 60,000 spores in 1 c.c. 25·6° C.

0·4 per cent.		0·7 per cent.		
Time in minutes	Per cent. germinated	Time in minutes	Per cent. germinated	Value of k
0	100	0	100	—
15	87·62	0·5	81·5	0·176
33	66·27	1	66·5	0·177
45	46·33	2	38·17	0·209
60	21·82	3	17·61	0·251
75	7·36	4	6·24	0·301
90	0·63	5·2	2·22	0·317
105	0·166	6	0·12	0·420
120	0·0	8	0·0	—
135	0·0			

will be seen that the experimental curve is not far removed from the normal. But if the 0·5, 0·6 and 0·7 per cent. curves are similarly transposed, the resulting frequency curves do not resemble normal curves. They are skew, the modal value has moved to the left, and moves further and further to the left, the greater the strength of phenol used (*c.* Fig. 9). If a logarithmic survivor curve were similarly transposed, it would give a "frequency curve," which has somewhat the shape of a reversed J. with the modal value on the extreme left.

This extreme J-form is a very unlikely form of distribution to find in an assemblage of Botrytis spores. Biological frequencies of this type are, of course, known, but they are very uncommon and we should not expect to find them here. This distribution is so unusual that those observers who regard the logarithmic as the typical disinfection curve reject (Chick, Eijkman, Arrhenius, etc.) the possibility of explaining it on any frequency basis whatever. They consider that though individual variations may be in part an explanation of departures from the logarithmic type, phenomena of quite a different category are responsible for the logarithmic type itself. Thus Chick in her first paper(3) looks on the bacteria as comparable to molecules with an internal cyclic revolution, and the interaction between poison and organisms as analogous to the inversion of sugar in presence of acid. She later transfers the interaction from the whole bacterium as such to the protein molecules it contains, and expressly rejects the possibility of resistance variations amongst the individuals having any significance when the reaction runs logarithmically. Arrhenius(13) says "there is no doubt that the individual cells in a sample of bacteria or red corpuscles possess a different power

of resistance to deleterious substances," but that the "different lifetime of the different bacteria does not depend in a sensible degree on their different ability to resist the destructive action of the poison."

This objection, however, rests on a misconception or confusion of thought, as has been pointed out by Brooks(11) already. In the frequency

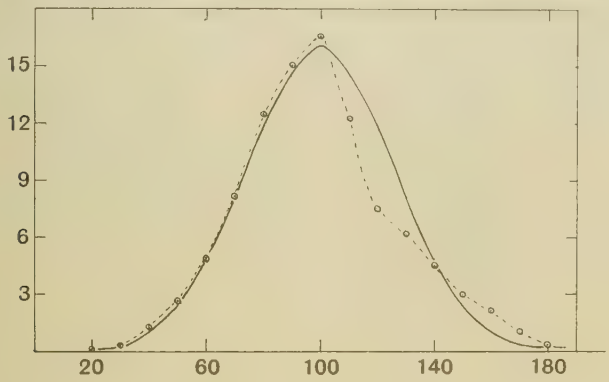

Fig. 8. Frequency curve derived from the 0·4 per cent. phenol sigmoid curve in Fig. 1, together with a normal curve (solid line).

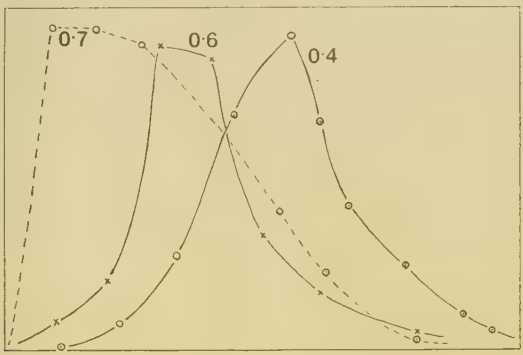

Fig. 9. Frequency curves derived from 0·4, 0·6 and 0·7 per cent. curves, showing increasing departure from normal with increase of phenol.

curve the base line represents resistance, increasing in grade or degree as one proceeds along it. *E.g.* if we were to suppose that the mechanism of resistance is simply the thickness of the spore-envelope, through which the poison has to penetrate, the base-line would represent increasing thickness measured in some selected unit. In the survivor curve our

only measure of resistance is the time taken to kill. But the times taken to kill of the survivor curve will correspond to the grades of resistance of the frequency curve, only if the reaction between spore and poison is proceeding at a uniform rate in all the grades. The two curves will agree only if it takes twice (3, 4 ... n times) as long to overcome 2 (3, 4 ... n) x grades of resistance as it takes to overcome x grades of resistance. They will correspond only if $R/T = k$, where R is resistance and T is time taken to kill. If this be not true, then it is illegitimate to translate the survivor curve directly into a frequency. If the reaction is proceeding faster in some grades than in others, if, *e.g.* it does not take twice as long for the phenol to go through a coat twice the thickness, but a time more or less than twice, then the times of dying do not accurately give the grades of resistance, and the true frequency curve can be derived from the survivor curve only by introducing some factor which will compensate for the change of rate.

This is the fallacy in the argument of those who object that the J-shaped frequency is an impossible form here. They assume tacitly and without evidence that the reaction is proceeding at a uniform rate in all the grades. But it has been shown above that for Botrytis spores at any rate this is not the case. As the strength of phenol is raised, the reaction runs quicker, relatively, in the spores which die early than in those which die late; and it is not a legitimate proceeding to transpose the survivor curve obtained with these high strengths of phenol directly into the frequency curve. What is got by so doing is not the real frequency curve. To get at the real distribution of resistances as they exist in the spores of the suspension it is necessary to introduce a compensating factor which allows for the change of rate.

What general form, applicable to all strengths of phenol, this factor should take, it is not possible at present to determine. The fact that we do get a nearly normal curve from the 0·4 per cent. survivor curve shows that, for that strength of phenol, the reaction is running at a nearly uniform rate in all the grades. If, however, with a sufficiently high strength of phenol, the change in R/T takes some such form as $R/\log T = k$, then we shall get from our original frequency curve a survivor curve that is nearly normal, and from the logarithmic survivor curve a frequency curve approaching normality.

To take an illustration: if we assume that the observed frequency curve shown in Fig. 8 accurately expresses the actual distribution of resistances in the spore suspension (it is not likely to be exactly accurate, because it is not likely that we have exactly hit off the correct strength

of phenol), then when $R/\log T = 50$, we obtain the following table of survivors at the times stated (Table XXI), and the survivor curve shown in Fig. 10. This shows no sigmoid character and approaches the logarithmic type. It is not strictly logarithmic, as is evident from the values of k given in the table; but if we had taken as our basis a true

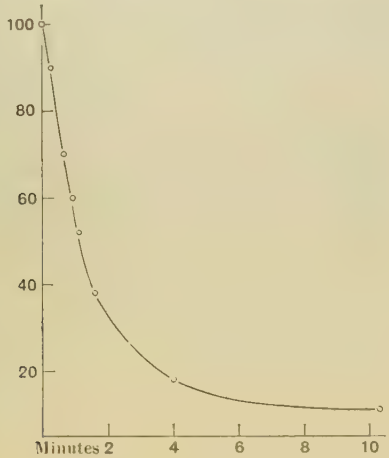

Fig. 10. Survivor curve obtained from the frequency shown in Fig. 8 by allowing for the change of rate with increased phenol.

normal curve, instead of the frequency obtained from the experimental observations, a much better approach to a true logarithmic curve would have been obtained. A strictly logarithmic survivor curve can result only if the frequency curve is slightly skew; but a survivor curve which in experiment closely simulates a logarithmic curve can be got from a strictly normal frequency.

Table XXI.

Time in minutes	Survivors per cent.	k
0	100	—
0·264	90·8	0·158
0·663	70·0	0·233
0·902	60·0	0·245
1·226	50·5	0·241
1·66	38·4	0·250
4·02	18·7	0·172
10·51	8·0	0·104
		Mean 0·200

Again, to take an illustration from anthrax spores as used by Chick: the observed logarithmic curve given by her (Table II, p. 99 in her first paper) is derivable from the frequency shown in Fig. 11 when $R/\log T = 55{\cdot}8$.

It is not suggested that the expression $R/\log T$ is necessarily the correct expression of the relationship between the times required to kill and the resistances. We have not sufficient data to arrive at a correct formulation of this relationship. But it is brought forward to show that by some expression of the kind, which takes into account the alteration in the rate of interaction between cell and poison produced by alteration in the strength of the poison, it is possible to obtain a logarithmic survivor curve from a frequency distribution of approximately normal shape, and that the occurrence of the logarithmic survivor curve does not compel us to look for its explanation in a direction entirely other

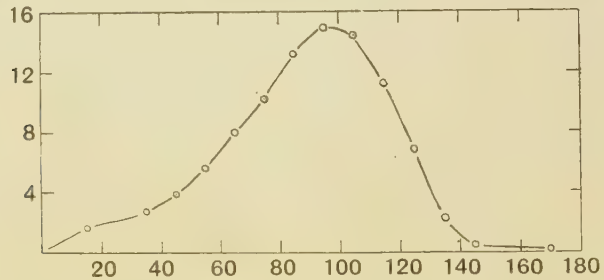

Fig. 11. Transposition of anthrax survivor curve (Chick) into frequency.

than that of differences in degree of resistances. The logarithmic and sigmoid curves are both dependent on the distribution of resistances in the suspension of spores or bacteria. They are both particular cases in the relationship between the resistance and the time required for killing; and may be regarded as different expressions of the same general principle, not as indicating phenomena of entirely different order.

It is not overlooked that this way of regarding the results has its own difficulties, the chief of which is that for a given distribution there is one value, and only one, of $R/\log T$, which gives the best approach to a logarithmic survivor curve. But the value of this expression may vary over a considerable range without any great departure from the logarithmic type, and the value of k in the formula $kt = \log a/a - x$ may also vary correspondingly. It also explains some facts which are otherwise difficult to interpret.

For example, we saw (Tables V, VI) that as we raised the amount of phenol from 0·4 per cent. to 0·7 per cent. the survivor curve tended more and more to assume the logarithmic shape. On reducing the temperature we were able to use 1·0 per cent. phenol, and yet the resulting curve was frankly sigmoid, and even with 1·25 per cent. the sigmoid character is still present. This is intelligible enough, if we consider that the relationship between resistance and killing time will be greatly modified by the lowering of the temperature; but it is difficult to understand if we suppose that the effect of the low temperature is merely to slow the reaction.

It seems clear that we must take into account the change of rate in different grades produced by the change of phenol strength: and it is a considerable simplification if, when this is allowed for, both the sigmoid and logarithmic curves can be derived from the normal or nearly normal distribution of resistances in the spores of the suspension.

I have pleasure in thanking Mr R. A. Fisher for assistance and criticism in the more mathematical aspects of this work.

Summary.

1. It is shown that if Botrytis spores be exposed to the action of 0·4 per cent. phenol, the spores do not all die simultaneously, but some die in a few minutes and some not till two or three hours have elapsed. The curve showing the numbers surviving at different times has a sigmoid shape.

2. If the strength of phenol be progressively raised, the curve becomes less and less sigmoid, approaching the logarithmic type of curve.

3. With the same suspension it is possible to obtain either a logarithmic or a sigmoid curve according to the strength of phenol used.

4. Both types of curve are shown to be explicable on the assumption that the individual spores differ in resistance and that a frequency curve showing the distribution in the resistance grades approaches the normal curve.

5. The influence of the number of spores used is shown to be very considerable; and the consecutive transition from the sigmoid to the logarithmic type occurs, whether we raise the phenol strength, keeping the spore number constant, or reduce the spore number keeping the phenol constant, or use younger and younger spores.

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(Further references on this subject may be found in the papers by Chick and Brooks.)

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BIOLOGICAL STUDIES OF *APHIS RUMICIS* LINN. 1746.[1]

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(With 1 Text-figure.)

Introduction.

In a previous paper (2), the author raised the question as to the influence of the different host plants on the reproductive capacity of *Aphis rumicis* and the resulting varying degree of infestation of different plants. From observations made on certain intermediate hosts of *A. rumicis*, it was found that some species of plants were more heavily infested than others, and an attempt has been made in the following experiments to obtain a numerical expression of the infestation of plants and of the relative susceptibility of the various hosts.

Experiments were carried out during 1913–14 at the Kaiserliche Biologische Anstalt für Land und Forstwirtschaft, Berlin, but owing to the war this series of experiments was terminated in July 1914 and, with the absence of the writer on service, it was not found possible to continue the work until the year 1920.

Some of the 1914 results (series A and B) are now given, together with certain of those for the season 1920 (series C and D).

In the 1914 experiments the aphids used were raised from ova. Ova of *Aphis rumicis* were found on *Euonymus europaeus* near Berlin, on March 25, 1914. These ova hatched out in the laboratory and the larvae of the Fundatrices were reared on *Euonymus* twigs grown under bell jars in wet sand. Some were then transferred to young *Euonymus europaeus* bushes grown in pots. The aphids used throughout the experiments were thus reared in captivity from the ova, through successive viviparous parthenogenetic generations as described below. Thus the

[1] A grant in aid of publication has been received for this communication.

history of the aphids used was known and also the exact generation with which the various plants were infected. Apterous viviparous ♀♀ were used for the infections, as winged forms are often at first restless on the plants, and it is difficult, when dealing with large numbers, to get a fair estimate of reproduction.

The plants were all grown from seed (except *Euonymus*)[1], and were kept covered with muslin bags throughout the experiments, to safeguard against outside infection. The aphids were transferred from one plant to another by means of a fine camel hair brush.

Unfortunately for the 1920 experiments, ova of *Aphis rumicis* could not be found, and the colonies in these experiments were started from an early viviparous parthenogenetic generation found on *Euonymus europaeus* on the laboratory farm on May 10, 1920. This generation was probably the second viviparous generation after the Fundatrix. In future experiments however, it is hoped to breed all the aphids from one egg and one Fundatrix.

It will be seen that the figures obtained in 1920, in general, support the results obtained in 1914.

The plants used in any particular series have as far as practicable been plants of the same age. They were grown in ten-inch pots and kept covered with muslin bags throughout the experiments. Unmanured soil, to which 10 per cent. of sand was added, was used. The plants were kept in a large glass house specially designed with large doors and windows. The great variation in temperature is a source of difficulty, since temperature is an important factor in affecting the development period and the number of aphids produced in a definite period of time. This has to some extent been overcome by arranging as far as possible that infections in each series of experiments were carried out on the same day. The temperature and humidity factors are thus relatively the same for each series of experiments. The temperature (daily, max. and min.) for the period June 25, 1920 to August 4, 1920 in the glass house is shown in Text-fig. 1 below.

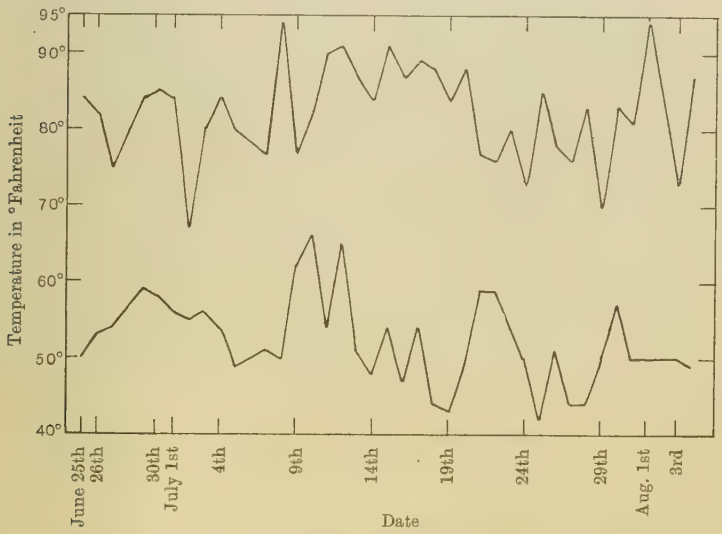
Series A. Infections from *Euonymus* to Different Plants.

(*a*) The aphids were reared throughout on *Euonymus europaeus* from Fundatrices, and the following plants infected with winged migrants of the 3rd v. gen.: broad beans, horse beans, poppies (*Papaver rhoeas*), peas, rumex, dwarf French beans and *Euonymus europaeus*. These plants were "stock" plants for infections to other plants of the same kind.

[1] My thanks are due to Messrs Sutton & Sons who supplied the seeds used in these experiments.

Three plants of each kind mentioned above were infected each with one a. v.[1] ♀, 4th v. gen. from its respective stock plant. After 14 days reproduction, all the aphids produced on each plant were counted.

Below is shown the progress of the infestation on these stock plants and Table I gives the number of aphids produced. The column "winged forms" in all the tables refers only to adults or advanced nymphs recognisable as immature winged forms.



Text-fig. 1. Max. and min. daily temp. of glass house between June 25th and August 4th, 1920.

Broad beans. A. 22. 5. 14. Infected with 9 w. v. ♀♀. 7. 6. 14. 4th v. gen. are all a. v. ♀♀; large size. 18. 6. 14. Many w. v. ♀♀ present; plant heavily infested.

Horse beans. A. 22. 5. 14. Infected with 3 w. v. ♀♀. 7. 6. 14. 4th v. gen. are all a. v. ♀♀. 18. 6. 14. Many w. v. ♀♀ present; plant heavily infested.

Papaver rhoeas. A. 22. 5. 14. Infected with 12 w. v. ♀♀. 9. 6. 14. Reproduction slower than on beans; only a. v. ♀♀ produced. 18. 6. 14. Moderate infestation, several w. v. ♀♀ present. 6. 7. 14. Plants sickly; aphids small size.

Peas. A. 22. 5. 14. Infected with 3 w. v. ♀♀. 9. 6. 14. 4th v. gen. few in numbers but fairly large size, all a. v. ♀♀. 29. 6. 14. Aphids moderate numbers, several w. v. ♀♀ present. 31. 7. 14. Aphids in moderate numbers, many a. v. ♀♀, small size.

Euonymus europaeus. A. 24. 5. 14. Infected with 4 w. v. ♀♀. 10. 6. 14. 4th v. gen. small numbers but fairly large size, all a. v. ♀♀.

[1] a. v. = apterous viviparous; w. v. = winged viviparous.

Rumex. A. 22. 5. 14. Infected with 3 w. v. ♀♀, 3rd v. gen. from *Euonymus.* 8. 6. 14. 4th v. gen. are all a. v. ♀♀; 17. 6. 14. w. v. ♀♀ present, infestation heavy.

Dwarf French beans. A. 22. 5. 14. Infected with 3 w. v. ♀♀. 8. 6. 14. Only 4 aphids alive, the w. v. ♀ mothers dead. 18. 6. 14. All the aphids produced on this plant are dead.

Table I.

Plant	No.	Date adult	Date aphids killed	Aphids produced: Winged forms	Aphids produced: Total
Broad beans	1	6. 6. 14	20. 6. 14	0	1143
,,	2	,,	,,	12	1154
,,	3	,,	,,	2	1192
Horse beans	4	6. 6. 14	20. 6. 14	15	1259
,,	5	,,	,,	13	1239
,,	6	,,	,,	0	902
Papaver rhoeas	7	9. 6. 14	23. 6. 14	5	243
,,	8	,,	,,	9	188
,,	9	,,	,,	16	128
Peas	10	9. 6. 14	23. 6. 14	0	37
,,	11	,,	,,	12	200
,,	12	,,	,,	0	189
Euonymus	13	17. 6. 14	1. 7. 14	2	28
,,	14	,,	,,	0	26
,,	15	Plant destroyed not going well			
Rumex	16	7. 6. 14	21. 6. 14	0	113
,,	17	,,	,,	0	135
,,	18	,,	,,	4	252

(*b*) Three plants of mangolds, red beet and sugar beet were infected, each with one w. v. ♀, 5th gen. from *Euonymus*, and one a. v. ♀, 6th v. gen. was left to reproduce on each plant. The number of aphids on each plant after 14 days are given in Table II.

Table II.

Plant	No.	Date adult	Date aphids killed off	Aphids produced: Winged forms	Aphids produced: Total
Mangolds	1	30. 6. 14	14. 7. 14	0	534
,,	2	,,	,, (Coccinellid larva present)	0	49
,,	3	,,	,,	0	206
Red beet	4	29. 6. 14	13. 7. 14	36	546
,,	5	,,	,,	0	17
,,	6	,,	,,	20	398
Sugar beet	7	29. 6. 14	13. 7. 14	67	622
,,	8	,,	,,	107	445
,,	9	,,	,,	115	696

SERIES B. INFECTIONS FROM *RUMEX* TO DIFFERENT PLANTS.

(*a*) Fundatrices of *Aphis rumicis* were transferred from *Euonymus* to *Rumex* and the aphids reared.

The following plants (to serve as stock plants) were then infected from *Rumex* with winged migrants of the 3rd v. gen.: broad beans, horse beans, poppies (*Papaver rhoeas*), peas, *Rumex*, and dwarf French beans. From these stock plants three uninfested ones of each of the above species were infected with one a. v. ♀, 4th v. gen., and the number of aphids produced in 14 days recorded. The following notes show the progress of the infestation on the stock plants, and Table III gives the number of aphids produced on the different plants.

Broad beans. R. 25. 5. 14. Infected with 3 w. v. ♀♀. 7. 6. 14. 4th v. gen. are all a. v. ♀♀, some adult, large size. 22. 6. 14. Infestation heavy, aphids small size.

Horse beans. R. 25. 5. 14. Infected with 3 w. v. ♀♀. 2. 6. 14. The w. v. ♀♀ produced nothing, so reinfected with 2 w. v. ♀♀. 11. 6. 14. 4th v. gen. in moderate numbers, large size, all a. v. ♀♀. 22. 6. 14. Infestation heavy, aphids small size.

Peas. R. 2. 6. 14. Infected with 5 w. v. ♀♀. 16. 6. 14. Moderate number of 4th v. gen., all a. v. ♀♀. 15. 7. 14. Many aphids are restless, the plant fairly heavily infested; many w. v. ♀♀ present.

Rumex. R. 24. 5. 14. Infected with 6 w. v. ♀♀. 10. 6. 14. 4th v. gen. all a. v. ♀♀.

Papaver rhoeas. R. 28. 5. 14. Infected with 3 w. v. ♀♀. 2. 6. 14. The w. v. ♀♀ produced nothing, so reinfected with 3 w. v. ♀♀. 15. 6. 14. 4th v. gen. are a. v. ♀♀; not so large as on beans; some adult. 6. 7. 14. Plant heavily infested; many w. v. ♀♀ present; aphids small size.

Table III.

Plant	No.	Date adult	Date killed off	Aphids produced: Winged forms	Aphids produced: Total
Broad beans	1	9. 6. 14	23. 6. 14	2	1465
,,	2	,,	,,	3	1418
,,	3	,,	,,	5	1457
Field beans	4	15. 6. 14	29. 6. 14	0	982
,,	5	,,	,,	8	1223
,,	6	,,	,,	6	1249
Papaver rhoeas	7	15. 6. 14	29. 6. 14	1	151
,,	8	,,	,,	2	82
,,	9	,,	,,	19	216
Peas	10	15. 6. 14	29. 6. 14	0	35
,,	11	,,	,,	4	34
,,	12	,,	,,	0	108
Rumex	13	10. 6. 14	24. 6. 14	0	35
,,	14	,,	,,	1	175
,,	15	,,	,,	0	147

Dwarf French beans not infected, reproduction poor.

Dwarf French beans. R. 25. 5. 14. Infected with 3 w. v. ♀♀. 10. 6. 14. Some aphids 4th v. gen. present, small size, scattered over plant, some dead; w. v. ♀ mothers dead. 15. 6. 14. Only five aphids alive, are a. v. ♀♀, very small size, three adult and producing 5th v. gen. 29. 6. 14. Very few aphids present, small size, dark brown colour.

(*b*) Mangolds, red beet and sugar beet were infected each with one w. v. ♀, 5th v. gen. from *Rumex*. Three plants of each kind were infected, and one a. v. ♀, 6th v. gen., offspring of the w. v. ♀ mother, was left to reproduce on each plant. The total number of aphids produced in 14 days was recorded as shown in Table IV.

Table IV.

Plant	No.	Date adult	Date killed off	Aphids produced: Winged forms	Aphids produced: Total
Mangolds	1	8. 7. 14	22. 7. 14	0	210
,,	2	,,	,,	0	87
,,	3	,,	,,	0	133
Red beet	4	6. 7. 14	20. 7. 14	0	143
,,	5	,,	,,	0	114
,,	6	,,	,,	0	202
Sugar beet	7	6. 7. 14	20. 7. 14	0	82
,,	8	,,	,,	0	238
,,	9	,,	,,	0	146

Series C. Reproduction of *Aphis rumicis* on ten varieties of Broad Beans.

The experiments of 1914 were continued in 1920, by series C and D.

As different varieties of broad beans might give a varying degree of susceptibility, one plant of each of ten such varieties was infected with an a. v. ♀ (offspring of winged migrant from the winter host) to form the stock plants for infecting others. All the seeds were sown on 3. 5. 20. Five plants of each variety were then infected with one a. v. ♀ (offspring of the apterous mother on the stock plant) from the respective stock plant. The apterous mother on each plant began producing on the 25. 6. 20, and the number of aphids after 14 days was counted, the total numbers being given in Table V.

The reproduction over 14 days on a further series of broad beans (Sutton's Prolific Longpod) was tested. The seed was sown on 26. 6. 2. The a. v. ♀ (taken from stock plant No. 6) on each plant, began to reproduce on 20. 7. 20. The results obtained are shown in Table VI.

Table V.

Plant	No.	Aphids produced: Winged	Aphids produced: Total	Plant	No.	Aphids produced: Winged	Aphids produced: Total
Sutton's Prolific Longpod	1	0	986	Sutton's Giant Windsor	31	0	1046
,,	2	0	937	,,	32	0	998
,,	3	0	934	,,	33	0	964
,,	4	0	844	,,	34	3	654
,,	5	0	782	,,	35	16	628
,,	6	stock plant		,,	36	stock plant	
Sutton's Improved Windsor	7	0	1230	Sutton's Green Giant	37	0	951
,,	8	0	1016	,,	38	4	865
,,	9	0	978	,,	39	2	745
,,	10	0	937	,,	40	0	733
,,	11	0	925	,,	41	21	591
,,	12	stock plant		,,	42	stock plant	
Sutton's Early Longpod	13	0	1021	Sutton's Early Mazagan	43	0	1269
,,	14	0	882	,,	44	5	1207
,,	15	3	783	,,	45	0	1106
,,	16	4	567	,,	46	0	987
,,	17	plant destroyed		,,	47	0	930
,,	18	stock plant		,,	48	stock plant	
Johnson's Wonderful	19	0	1028	Dwarf Royal Cluster	49	4	947
,,	20	0	970	,,	50	5	857
,,	21	0	910	,,	51	10	720
,,	22	0	795	,,	52	3	698
,,	23	Syrphid larva present	383	,,	53	2	510
,,	24	stock plant		,,	54	stock plant	
Taylor's Windsor	25	0	974	Beck's Dwarf Green Gem	55	2	1290
,,	26	0	901	,,	56	3	977
,,	27	7	876	,,	57	3	953
,,	28	0	716	,,	58	3	781
,,	29	0	676	,,	59	mother died early	90
,,	30	stock plant		,,	60	stock plant	

Table VI.

Plant	No.	Aphids produced: Winged	Aphids produced: Total	Plant	No.	Aphids produced: Winged	Aphids produced: Total
Sutton's Prolific Longpod	1	0	1010	Sutton's Prolific Longpod	6	0	537
,,	2	0	886	,,	7	2	515
,,	3	0	833	,,	8	0	490
,,	4	3	629	,,	9	5	361
,,	5	0	552	,,	10	0	47

Series D. Reproduction of *Aphis rumicis* on Mangolds, Sugar Beet, etc.

One pot each of mangolds, sugar beet, red beet, poppies, peas and dwarf French beans (seed sown 3. 5. 20) was infected with two a. v. ♀♀ from broad beans on 9. 6. 20. These were the stock plants from which four uninfested ones of each species (seed sown 3. 5. 20) were infected with one a. v. ♀. The number of aphids produced in 14 days was recorded, and the reproduction figure for each plant is shown in Table VII. The progress of the infestation of the stock plants is given below.

Table VII.

Plant	No.	Date adult	Date killed off	Aphids produced: Winged	Aphids produced: Total
Sugar beet, Klein Wanzleben	1	29. 6. 20	13. 7. 20	0	156
„ „	2	29. 6. 20	13. 7. 20	0	141
„ „	3	29. 6. 20	13. 7. 20	5	130
„ „	4	29. 6. 20	13. 7. 20	3	66
Sugar beet, Carter's No. 1	5	3. 7. 20	17. 7. 20	0	294
„ „	6	1. 7. 20	15. 7. 20	0	119
„ „	7	3. 7. 20	17. 7. 20	0	73
„ „	8	1. 7. 20	15. 7. 20	0	58
Red beet	9	3. 7. 20	17. 7. 20	0	197
„	10	2. 7. 20	16. 7. 20	0	164
„	11	3. 7. 20	17. 7. 20	5	85
„	12	2. 7. 20	16. 7. 20	10	16
Mangolds	13	1. 7. 20	15. 7. 20	0	201
„	14	30. 6. 20	14. 7. 20	3	177
„	15	30. 6. 20	14. 7. 20	10	155
„	16	30. 6. 20	14. 7. 20	0	42
Poppies, Shirley	17	10. 7. 20	24. 7. 20	4	193
„	18	7. 7. 20	21. 7. 20	2	178
„	19	9. 7. 20	23. 7. 20	25	161
„	20	8. 7. 20	22. 7. 20	33	123
Euonymus	21	1. 7. 20	15. 7. 20	16	117
„	22	30. 6. 20	14. 7. 20	16	97
„	23	30. 6. 20	14. 7. 20	0	20
„	24	30. 6. 20		Aphid mother died	

Mangolds. E. (Carter's Red Chief.) 19. 6. 20. Infected with 2 a. v. ♀♀ from broad beans (offspring of winged migrants on beans). 30. 6. 20. A few aphids produced (three w. v. ♀♀, remainder a. v. ♀♀).

Sugar beet. E. (Carter's No. 1 and Klein Wanzleben.) 19. 6. 20. Infected as above. 29. 6. 20. Two w. v. ♀♀, remainder a. v. ♀♀, some adult and producing next generation.

Red beet. E. (Carter's Perfection.) 19. 6. 20. Infected as above. 30. 6. 20. A few aphids produced (one w. v. ♀, remainder a. v. ♀♀, some adult).

Poppies. E. (Shirley.) 19. 6. 20. Infected as above. 29. 6. 20. Aphids going poorly, small size, plants poor. 5. 7. 20. Aphids now going well, many immature winged forms.

Peas. E. (Carter's Laxtonian Dwarf Pea.) 19. 6. 20. Infected as above. 25. 6. 20. A small colony going; killed off the apterous mothers. 30. 6. 20. Twenty aphids present; small size; three a. v. ♀♀, remainder w. v. ♀♀ (not adult). 14. 7. 20. Both the a. v. ♀♀ and w. v. ♀♀ have produced young. 29. 7. 20. Aphids along mid ribs of leaves, developing slowly, small size. 2. 8. 20. Killed off the aphids, 5 a. v. ♀♀, many immature w. v. ♀♀.

Dwarf French beans. E. (Carter's Canadian Wonder.) 19. 6. 20. Infected as above. 2. 7. 20. Twenty aphids present, scattered over the plant, small size, several dead, one a. v. ♀ adult. 5. 7. 20. Three a. v. ♀♀ adult, very small size, producing young. 26. 7. 20. Aphids increasing in numbers, very small size, all a. v. ♀♀. 28. 7. 20. Plant in flower, aphids on flower heads and young seed pods, slightly larger size. 2. 8. 20. Killed off the aphids; small size; all apterous forms.

Euonymus. E. 16. 6. 20. Infected as above. 29. 6. 20. Moderate number of aphids produced, a. v. ♀♀ and w. v. ♀♀. 20. 7. 20. Many w. v. ♀♀.

General Discussion.

(*a*) *Influence of Food Plants on Reproduction.*

It will be seen from the foregoing data that, given a favourable food plant, and favourable conditions of temperature and humidity, rapid reproduction will result. A comparatively high reproduction figure with rapid development was maintained on broad beans, while on peas, mangolds, sugar beet, red beet and poppies, the figures were considerably lower and the progress of the infestation was somewhat slower.

On dwarf French beans in 1914 the aphids fared very badly, only a few young were produced and the colony eventually died out. Again in 1920 on the variety Carter's Canadian Wonder, the aphids progressed very slowly and poorly, many died and those which remained alive developed into small, dwarf individuals, producing only a few young. However, it was possible to keep them going on the plant from 19. 6. 20 to 2. 8. 20, at which date the plant was flowering, some of the aphids on the young developing pods making a little more progress. The experiment was stopped on 2. 8. 20.

Similarly on peas in 1914 the reproduction figure was low and the development period retarded, although, after two months, a number of aphids were found.

In the 1920 experiments, on dwarf peas (Carter's Laxtonian Pea and Carter's Little Marvel), a few aphids of small size were produced and the development period was prolonged.

Colonies of *Aphis rumicis* have been recorded on scarlet runners (Davidson(2)), on climbing haricot beans (Malanquin and Moitié(6)) and many other plants, where the infestation is not heavy as compared with beans. There is a wide distribution of the species on different kinds of plants which are less or more favourable as indicated by the degree of infestation.

The food of aphids is the sap derived from certain cells of the host plant. Sections of plant tissues fixed with aphids *in situ*, show that the phloem of the vascular bundles is particularly sought after, though cells of the cortex, parenchyma and mesophyll of the leaf, are also tapped by the stylets. This question is being further studied, but it is evident that while the phloem is very important other cells of the plant must be regarded as a source of nourishment.

The constitution of the cell sap may differ considerably in different species, and the hydrogen-ion concentration of cell sap should be investigated in relation to the relative susceptibility of plants to aphid attacks. In this respect Comes'(1) view that the degree of acidity of the cell sap of American vines is in direct relation to the resistance to Phylloxera is important, as is also the recent work on the hydrogen-ion concentration of plant juices and the factors affecting it.

Since physiological characters of plants may be specific and follow Mendelian laws of heredity, this line of investigation, with the possibility of breeding resistant strains, would seem to be a profitable one, and research as to the relative intensity of reproduction on different varieties of the same species is being continued.

Since the chemical composition, or physiological characteristics of the cell sap, is an important factor, one is inclined to the view that the various species of aphids, at any rate in the viviparous parthenogenetic generations, have adapted themselves to many different plants.

In the wide distribution of winged migrants, and the fortuitous adventures of these individuals, they may alight on many different plants—especially in the case of a marked polyphagous species like *Aphis rumicis*—and produce young. Certain plants (more susceptible) provide the necessary stimulus for rapid reproduction. On other plants on the contrary only a moderate reproduction occurs, and on others the colonies may soon die out.

The adaptability of the aphis to its food plants is an important consideration, and is doubtless influenced by the nature of the food plants available. This is a local consideration and probably accounts for the widely different food plants (cultivated or otherwise) to which species

of aphids have adapted themselves in different countries; this being an expression of local races and local conditions. For instance, although my figures of reproduction on sugar beet are comparatively low, it is feasible to expect that, in districts where sugar beet is the most widely distributed favourable food plant, this reproduction figure may be higher. In this respect the reproduction figures on sugar beet for 1914 and 1920 are interesting. To quote an example: Davis (1909) has shown that *Aphis maidis* Fitch, prefers broom corn to Indian corn or sorghum. In the struggle for existence against adverse conditions, the chances of survival are greater for the strain of aphis produced on plants which cause a high reproduction figure.

It seems probable, although the data obtained from my experiments are not sufficient to draw conclusions, that reproduction on certain intermediate hosts may be affected by the nature of the previous host on which the aphids were reared. In Tables II and IV it will be seen that mangolds, red beet and sugar beet, infected with a strain of *Aphis rumicis* which had been reared for several generations on *Euonymus europaeus*, gave a higher reproduction figure than when infected with a strain reared on *Rumex*. Similarly, higher figures were obtained on these plants when they were infected with strains of *Aphis rumicis* reared on beans and poppies. This question, however, requires further investigation with reference to the factors concerned, especially the condition of the plant and temperature conditions.

There are the further questions of the influence of climate, varying soil conditions, agricultural methods, and the manurial treatment of crops, on the constitution of the cell sap of plants. The importance of manurial treatment in relation to the susceptibility of varieties of wheat to attacks of rust fungus is well known. According to Comes, nitrogenous fertilizers stimulate the cells of the plants, causing the tissues to be more juicy and diminishing the acidity of the cell sap.

The ten varieties of broad beans experimented with (Table V) showed little difference in the degree of susceptibility to attacks of *Aphis rumicis*. One might infer that the varieties Sutton's Improved Windsor and Early Mazagan are slightly more susceptible than the other varieties, and that Dwarf Royal Cluster, Sutton's Green Giant and Taylor's Windsor are slightly less susceptible. It is clear however that these strains of beans are too closely allied to give any striking difference. In any case, they only represent a very limited number of the varieties obtainable. Experiments are being continued with varieties of field beans.

The wide range of variation in the numbers on each variety, is probably largely due to the variable fertility of the agamic females.

In August and September 1913, 30 a. v. ♀♀, offspring of wild w. v. ♀♀ of *Aphis rumicis*, were isolated on broad bean in a greenhouse, with a temperature varying from 62° F. to 85° F. The daily reproduction and total offspring produced by each mother were recorded. The maximum number of young born in any one day was 8. The minimum being 1. The maximum total number produced was 45 and the minimum 6. Three examples are given showing a low figure, a medium figure and a maximum figure of reproduction.

Temp.° F.	62	67	77	70	77	77	85	80	70	77	65	70	65	Average daily production	Total aphids produced
August, 1913	19	20	21	22	23	24	25	26	27	28	29	30	31		
Plant 1	0	0	3	1	2	1	0	3	2	0	0	0	—	1·7	12
,, 2	6	2	2	6	0	1	0	1	0	2	0	0	—	2·0	20
,, 3	5	5	2	5	2	5	3	4	4	4	6	0	—	4·1	45

It should be noted, however, that these aphids were taken towards the end of the season, and the history of the previous generation was not known.

There would appear to be some relation between the time of the year and the degree of infestation of plants.

Recorded observations of marked decrease in the reproduction of aphids during high summer temperatures, indicate an association of temperature and the season of the year with the "dryness" of food plants as contrasted with the young succulent growth of spring and early summer. It seems feasible to expect as a result of these factors, a seasonal adaptation of aphids to the varying seasonal conditions. The number of the viviparous generation used in experiments may therefore be an important consideration when results obtained at different periods of the season are compared.

In my experiments, there was a marked decrease in the numbers of aphids produced towards September and October, probably to some extent due to lower temperature conditions and to the appearance of sexual forms in the cultures; but cultures, carried on throughout winter in a warm greenhouse, also produced few aphids in comparison with the reproduction in June. To some extent the appearance of sexual forms in each generation accounts for this, but my observations indicate that the greatest reproduction occurs with the early viviparous generations.

Experience in breeding aphids shows that in investigating the reproduction on different hosts, the following factors must be considered:

(*a*) The physiological condition of the plant, especially with regard to its age, temperature, light and food.
(*b*) Temperature and humidity conditions.
(*c*) The history of the previous generations, the generation of the aphids used, and the kind of plants on which they have been reared.
(*d*) Whether winged or apterous agamic females are used.
(*e*) The variability in reproductive power of the individual agamic females.

(*b*) *Influence of Food Plants on the Characters of the Species.*

The quantity and quality of the food are very important factors in affecting the appearance of *Aphis rumicis*, especially in regard to colour and size. When kept for several generations on *Euonymus* the aphids in the later generations are very much smaller than the earlier generations, but by cutting back young trees, thus producing a succession of young growth, the aphids were carried on the spindle tree for several months. They were somewhat smaller than the earlier generations, and considerably smaller than the corresponding generations on broad beans, also the reproductive capacity on *Euonymus* was greatly inferior. On broad beans the aphids were always large, healthy individuals, except in the case of very heavily infested plants. On poppies they were somewhat smaller. On mangolds, sugar beet, and red beet they were also smaller than on beans. On dwarf peas they were quite small individuals but healthy in appearance, while on dwarf French beans they were extremely small, dwarf individuals with very low producing power.

There is in a general way some correlation between the size of the individuals on the host plant, and the number of aphids produced. This correlation between size and fertility is also indicated in Warren's[7] statistical examination of *Hyalopterous trirhoda* Walker.

Ewing[3] in his interesting statistical study of *Aphis avenae* Fab. has shown the great range of fluctuating variations over a number of parthenogenetic generations, these variations being individual and not inherited. Extremely high and low variations were traced to the effects of food and temperature conditions. Temperature will of course also indirectly affect the food factor, in so far as it influences the metabolism of the plants.

With *Aphis rumicis* it was found that small agamic females from dwarf French beans, when transferred to beans, produced young which developed into normal size.

(*c*) *The Influence of Temperature and Humidity on the Development of the Species.*

It was observed throughout the experiments that low temperatures retarded the development period and the production of young over a given period. For this reason figures of reproduction for one period cannot be safely compared with those for another period, unless due regard is paid to temperature conditions. Further it appears probable that the maximum reproduction occurring in early summer, with a decreasing reproduction later in the season, may be an expression of adaptation, and that even with favourable conditions of food and temperature with later generations, later in the year, the high reproduction figures of earlier generations are not obtained. It is interesting to compare Tables V and VI in this respect. Recorded observations show that there is a decrease in the number of aphids during high summer temperatures and experiment demonstrates that abnormally high temperatures restrict the development: it has been pointed out above, that the association of high summer temperatures with the "dryness" of the food plants at that period, involves the factor of food conditions with a possibility of a seasonal adaptation of aphids.

Aphis rumicis could not be reared in winter in the warm dry air of the laboratory, most of the young dying before reaching maturity; whereas in a similar temperature in the humid atmosphere of the greenhouse they developed normally.

Headlee(4), found with *Toxoptera graminum* Rondani, that with a constant humidity of 75 per cent., the development period from birth to maturity varied according to the temperature. Variations in atmospheric humidity (between about 37 per cent. to 100 per cent.) did not have any marked effect on the metabolism of the aphids. Similarly variations of 20 degrees or so about the optimum temperature did not produce any marked effect.

Klodnitski(5) also showed the retarding effect of low temperatures on other species of aphids. It would appear that there is an optimum temperature for development which may vary for different species.

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SOME RELATIONSHIPS OF ECONOMIC BIOLOGY[1]

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BEING THE PRESIDENTIAL ADDRESS DELIVERED TO THE ASSOCIATION OF ECONOMIC BIOLOGISTS AT THE ANNUAL GENERAL MEETING, FEBRUARY 18, 1921

I have to thank you for the opportunity of presiding over our deliberations during the past year. Your consideration and the kindness of my colleagues on Council have made the privilege a pleasure. That pleasure would have remained unmixed but for a reminder that to-day is the occasion for an annual address.

Our Secretary in making this intimation has kindly added that although such addresses usually deal with the wider issues of biology there is no established precedent which need be followed. Grateful for this assurance I will restrict my remarks to some outline of the varying relationships which our work as economic biologists has borne to the activities by which natural knowledge is gained.

It is convenient to consider separately the philosophical relationship between economic and natural study, and the development of that practical relationship familiar to those whose personal recollections go a generation back.

As economic work antedates philosophical study it would seem proper to consider the practical relationship first. But it is more convenient to reverse this order. In this country one philosophical relationship between economic and natural study was clearly defined by the middle of the seventeenth century, and the evolution of that conception helps us to understand why effect was not given to it till our own day.

Our Association is concerned with Economic Biology. Economic Biology is conditioned as other natural studies are. Its business is to deal with natural things. These assort themselves into two groups. Regarding most things all we can say is that they exist and that observation and experiment have revealed some of their effects. Only

[1] A grant in aid of publication has been received for this communication.

as regards a few may we venture to imagine that we understand their causes or know their nature.

The economic worker shares with the seeker after truth for truth's sake the hope of detecting natural effects hitherto unsuspected. He may even aspire to increasing the number of known natural things. If his duties debar him from attempts to explain natural causes, they at least afford him reason for wishing that his knowledge were less circumscribed than it is.

The existence in divers degrees of these hopes and wishes led to the old subdivision of students of nature into those who are "learned" and those who are "curious." Both rely on the aid of the same two studies: History, which records what is known; and Philosophy, which considers what may be knowable. Both depend for success on the same two faculties: Observation which, where fully trained, occasionally perceives what is obvious; and Imagination which, when properly controlled, sometimes suggests how knowledge may be sought.

The "learned" are largely incited to study by the effects of natural qualities. They keep imagination under control. Their progress both in conception and in accomplishment is *per gradum*. The "curious" are more attracted towards enquiry into natural causes. They use imagination to devise theories. If the conception which gives rise to a hypothesis and to the experiment which tests it be philosophical, the progress, if any, which they make is *per saltum*. Whatever may be the case in other studies Economic Biology is beholden to both.

The peculiar merits of each sometimes develop into defects. If the "learned" fear to employ imagination their progress is slight. If they rely implicitly on authority their progress may cease. If the "curious" become the victims of imagination they may lose ground by accepting explanations of natural causes which neither observation nor experiment can confirm.

Philosophical study has been most impressed by these defects. The term "learned" has been relegated to literature; the term "curious" has fallen out of use. The pejorative significance which these names have acquired among students of nature has its disadvantages. The economic biologist has reason to know that the discontinuance of this classification has neither altered the conditions that led to its adoption, nor modified the circumstances that dictated its abandonment. He is, however, left to discover, after he has embarked on economic study, how valid and vital the old distinction was.

Yet academic natural study was perhaps justified in discarding these

terms. She thus disavowed disciples wedded to authority or satisfied with conjecture. She encouraged philosophical observation and experiment by abandoning systems of nature founded on unconfirmed statements and natural laws based on unverified hypotheses. She placed natural enquiry on a sounder basis. She realised that the promotion of natural knowledge is a means to that higher end, its application for use or for further discovery. As natural things were put to practical use before the study of natural effects was undertaken, the acceptance of the novel philosophical principle that the application of natural knowledge is a human duty, was at the same time a recognition of an established historical fact.

When the habit of noticing natural effects began there was little tendency to enquire into natural causes. This was not because the latter were overlooked; it was owing to a belief that natural causes are explicable by the principles of sympathetic magic which are assumed though never defined by savage races and are still at times subconsciously accepted by races that claim to be refined.

A change takes place when the perception of natural effects is supplemented by spiritual conceptions. Revelation, which faith accepts, is antithetic to the principles of sympathetic magic. It undertakes to do more than these principles can. Sympathetic magic only explains the causes, revelation professes to explain the effects of natural qualities as well. The explanation is less precise. But it is more comprehensive and effectively inhibits natural curiosity. The only human interest which revelation does not always destroy is that in the "virtues" of things.

Some faiths have been helped by specialisation in this interest. Their leaders have found it profitable to cultivate an expert acquaintance with natural effects. The reflected spiritual quality thus imparted to them has at times prolonged sacerdotal authority over the vulgar when profane study has left the priest relatively imperfectly informed.

Most spiritual laws find their fulfilment in new faiths. A fresh spiritual impulse as a rule succeeds more rapidly if its pioneers possess miraculous powers. Where these powers are extensive, the new faith regards the application and the promotion of natural knowledge as equally uncalled for, and the applied worker finds his occupation gone.

Grace, however, may diminish as influence grows and in one familiar case, where spiritual authority eventually brought temporal power under control, the grace failed to protect the State against external aggression. This accident modified spiritual attitude towards natural knowledge. Promotion of natural knowledge remained an offence; the use of natural

knowledge was re-sanctioned and the applied worker once more came into his own.

We may regret the restriction. We have, however, to admit that the treatment accorded to natural study and spiritual discussion was strictly impartial. Dogma became the substitute for both and remained in operation for a millennium.

When effective objection to dogma eventually arose, the protest against the spiritual variety found greatest popular support. But the less articulate revolt against natural dogma was that whose effects have been most enduring. In some countries the desire for spiritual emancipation was slight. There ecclesiastical authority encouraged natural enquiry, provided the results published did not controvert scripture. In lands where a reformed spiritual doctrine became dominant the tendency was to make the embargo on natural study more stringent. Protestant conviction emulated primitive faith in its condemnation of profane efforts to apply natural knowledge for the good of the community.

Few of those Governments that with the help of popular antagonism to spiritual dogma had thrown off one ecclesiastical yoke were minded to subject themselves to another. They therefore encouraged the promotion of natural knowledge on the understanding that any new knowledge secured be improved in the public interest.

The application of natural knowledge thus encouraged by the State and assented to by philosophy, had as its basis the promotion of such knowledge. The promotion of natural knowledge involves two operations: the search for further truth and the co-ordination of new facts with pre-existing knowledge. The search for truth is a constructive activity involving original enquiry. The co-ordination of knowledge is a critical activity which may reveal the need for research into subjects hitherto accepted as explained. Enquiry and research are only preliminaries to consideration as to how far and in what way their results may be improved. In applying such results it was agreed that improvement for use take precedence over improvement for discovery.

Application of knowledge for use takes two forms. The knowledge may be employed to promote intellectual development or to supply material needs. The former usage gave rise to studies once known as "moral history" and "moral philosophy," the latter to studies termed "natural history" and "natural philosophy." The first of these terms has disappeared; the others are still familiar, though in all the significance of the term has changed.

Economic biologists have to read old books as well as new. They therefore know that early naturalists wrote "Moral and Natural Histories," dealing with "everything performed by man or produced by nature" in particular regions. This union by the field-worker of subjects which the scholar can keep apart is due to the community of interest of "moral" and "natural" study in the "qualities" of things. The "moral historian" deals primarily with the "uses" dependent on the existence of definite "qualities" whose presence compels the "natural historian" to detail the "characters" of the things in which they are manifested. The "economic naturalist" must attend both to the "characters" and the "virtues" of things, the latter being the term employed to include both "qualities" and "uses."

Economic study is thus something complete in itself, which also links "moral" and "natural" history. Hence the evolution of that alternative scheme of knowledge wherein the various philosophical and historical studies were aggregated as "sciences," while the activities concerned in the application of knowledge were subdivided in terms of the underlying purpose. The agencies charged with the application of knowledge in doctrine were designated "letters"; the technical pursuits involving the application of knowledge in practice were styled "arts." In this brotherhood of the "Sciences, Letters and Arts," whose foundation was contemporary with the evolution of the philosophical scheme for the "improvement of natural knowledge," "application" was collateral with instead of consequent upon "promotion" of knowledge.

"Natural history" and "natural philosophy" at first bestowed appropriate attention upon the "characters" and "virtues" of all natural things. Soon, however, both felt the influence of the distinction between things organic and things inorganic. By degrees "natural philosophy" concentrated her attention on the inorganic, leaving the organic to be dealt with elsewhere. This habit led "natural philosophy," by an easy transition, to the study of physical and chemical problems, leaving those problems connected with organic structure and function to be dealt with by the correlated economic agencies. When "natural philosophy" took cognisance of physiological problems at all she did so because these problems, though of biological origin, were "statical" in character. This practice reacted on "natural history" whose duty is to codify and co-ordinate natural knowledge and to co-operate with "natural philosophy" in applying it. The understanding tacitly reached was that "natural history" should advance as well as co-ordinate knowledge regarding the characters and virtues of organisms, while "natural philo-

sophy" undertook to co-ordinate as well as to advance physical and chemical truth. A subdivision of natural study according to subject thus replaced one according to method.

This change was convenient. Its only disadvantage was the retention of a terminology no longer appropriate. History is of equal consequence to physics and to biology; co-ordination of knowledge is as essential to inorganic as to organic study. Biology makes use of philosophy just as physics and chemistry do. If philosophy enabled physics to establish the principle of "conservation of energy," she helped what we still term "natural history" to formulate the doctrine of "evolution."

The new arrangement was already recognised before the end of the first quarter of the eighteenth century and "natural history," as then understood, has observed her obligation ever since. The effect of the change on economic biology is what interests us now. Our study obtained from "natural history" that philosophical acquaintance with the characters and virtues of animals and plants as organisms which underlies "domestication." For information regarding the structure and functions of the animal and the plant as mechanisms, economic biology was indebted to the Institutes of Medicine, already a powerful technical study, and the Institutes of Rural Economy, one still relatively backward.

By the middle of the eighteenth century the mechanical arts decided to follow the example of medicine. Aware of the progress due to technical studies evolved by the art of healing, "natural philosophy" approved the adoption by the engineer of technical in place of philosophical methods in securing the knowledge he required. This enabled "natural philosophy" to limit her activities to the promotion of inorganic natural knowledge. Relief from the task of applying knowledge induced an increase in the activity involved in codification. This new impulse spread from inorganic to organic philosophical study and natural knowledge underwent an "encyclopaedic" phase. The more intensive interest in the characters and virtues of animals and plants thus induced was not accompanied by, and is thought by some to have inhibited, a comparable activity in the study of organic structure and function. During this period therefore the relationship of economic biology to natural study was hardly affected.

Among the tasks undertaken by physical and chemical technique at the instance of the mechanical arts were those of providing biological study with instruments more effective and methods more precise. The success which attended these efforts was such that within the first

generation of the nineteenth century, philosophical enquiry into animal-structure and plant-structure enabled "natural history" to co-ordinate knowledge and "economic study" to apply it with an assurance hitherto impossible. The technical study of function on the nutritive side had taught medicine that health and disease are opposite faces of one shield, and had satisfied husbandry that plant-growth is more than a mechanical process. Pathological technique now told the physician how to diagnose; chemical technique felt justified in teaching the farmer how to till. Such techniques received academic recognition and were raised to the status of studies comparable in importance with "natural history," which now underwent fission into branches restricted respectively to animals and vegetables. But while in both cases the study of organic structure could now be linked with the earlier interest in the characters and virtues of living things, neither branch of "natural history" undertook the philosophical study of organic function.

The philosophical study of reproduction had not yet set in; the technical study of this subject, which played so important a part in the early history of mankind, had degenerated into a traditional empiricism. The technical study of nutritive function was shunned by both branches of "natural history" for two rather different reasons. The study of animal nutrition was the prescriptive right of medicine; the study of plant nutrition from the standpoint of the substratum was so ardently pursued by chemistry that the part played by the plant itself hardly interested husbandry.

The academic incorporation of techniques older than their correlated philosophical studies afforded evidence of a deeper change. The seventeenth century interest in the improvement of natural knowledge had been narrowed in the eighteenth through the delegation to technical study of the duty of applying such knowledge. The eighteenth century interest in the promotion of natural knowledge had been further narrowed in the nineteenth by the exaggeration of the distaste for "encyclopaedism" into an aversion towards systematic work. Academic interest was concentrated upon the philosophical increase of natural knowledge and its application in doctrine. These two activities were now amalgamated in the comprehensive term "natural science."

Every old technique has been the product of some correlated art, at whose service the activities of the technique were placed. The co-ordination and application of the knowledge gained were duties appertaining to the art concerned. Yet every sound technique eventually reaches a stage when it seeks new knowledge on its own initiative. The

correlated art still codifies and employs the knowledge thus supplied. But the technique now puts this new knowledge to doctrinal use by instructing the art it serves.

Technical and philosophical studies thus move in opposite directions until a point is reached at which they enjoy community of interest. Alliance is inevitable. Equally natural is the adoption of the view, characteristic of the latter half of the nineteenth century, that the "advancement of science" is the only activity which deserves academic distinction.

Academic distinction, however, even at the close of last century, was not the only incentive to philosophical study. Some there were who regarded the search for truth as its own reward. With others the public good was a sufficient stimulus to effort. Enquiry into the effects of things organic, which chiefly concerns us, was thus, a generation ago, undertaken by three distinct agencies: Natural History, directed to the promotion of knowledge by increasing and co-ordinating it, as a preliminary to considering how best it may be improved; Academic Science, engaged in the advancement of natural knowledge, with a view to its employment for discovery and doctrine; and Applied Biology, occupied in seeking new and sifting old knowledge, with the object of furthering economic ends.

Economic Biology endeavours to serve the various human arts as their own old technical studies did. But that service is now rendered *solute nec abjecte*, and the new technologies devised to carry it out derive their methods from Academic Science and their inspiration from Natural History.

There need be no antagonism between agencies whose outlooks differ. Any misunderstandings that may occur find their explanation in a pardonable inability to appreciate unfamiliar points of view. The natural historian must regard his facts from the standpoint of their bearing on the duties of his study to natural knowledge as a whole; the academic enquirer may concentrate his attention on the new facts he secures; the economic biologist must regard his facts from the standpoint of their value to the industrial interests he desires to assist.

The great impediment to cordial intercourse between Academic Science and Natural History during the closing generation of last century was the obstinacy with which the latter agency still pursued the always difficult and often thankless tasks of determination and classification. The Natural History of Animals was allowed to retain academic status under the style and title of "Comparative Anatomy,"

on the understanding that philosophical interest in the characters and structure of the subjects of study be discreetly veiled. But the Natural History of Plants was formally debarred from academic intercourse and the philosophical study of nutritive plant-function has been installed in its stead. The sincerity of this objection to co-ordination is best revealed by the inconsistency of academic attitude towards the philosophical study of plant-structure. For a time this study shared, on terms of equality with plant-physiology, the position of which Natural History has been deprived. Unfortunately the facts of plant-structure lead to advances in philosophical classification and the study of plant-structure has become so suspect that it is now the fashion to declare that the truths of vegetable morphology are unprofitable for purposes of doctrine. But while Academic Science has to some extent endorsed this view, the "Comparative Anatomy of Plants" has been tacitly conceded certain academic privileges. The plant-morphologist may still continue in the palaeontological field the philosophical tasks of reconstructing vanished vegetations and determining geological horizons. Academic Science has also made an exception in favour of the morphological technology which endeavours to explain the causes of those nutritive functions whose effects interest the plant-physiologist, and attempts to demonstrate the mechanism of those reproductive functions whose effects extend to the domain of moral study.

Economic Biologists experience no difficulty in appreciating the determinative interest of the Natural Historian. We benefit at every turn from the results of historical study regarding the characters and virtues of organic entities, and are glad of the assistance the naturalist can give as regards the philosophical limitation of the essences with which we deal. The ardour of the Natural Historian in systematic study has placed Economic Biology under further obligation: "Plant Pathology" has been evolved from the natural histories of invertebrate creatures and cryptogamic plants, therein differing from "Animal Pathology" which is a bye-product of Animal Physiology. We have, as Economic Biologists, a stronger bond of sympathy still with the Natural Historian. We have discovered in the course of our own work that in the soil we have a fauna and a flora as complex and as important as those that appear upon it. We realise that our first business is to codify this new knowledge. In accomplishing this task we may find it expedient to adopt methods of our own. But we appreciate already that we may encounter difficulties with which Natural History is familiar, and that we must attack our problem in her spirit.

Economic Biology has reason to know that the interdependence of Natural History and Academic Science is equally marked. Oecology, whose results we apply to everyday affairs, has shown us that diversity of purpose is no barrier to community of interest. Economic Biology realises, even if Academic Science and Natural History do not, that the field-naturalist cannot escape being an Oecologist, and that the Oecologist is a Natural Historian in spite of himself.

Notwithstanding the suspicions she has aroused, Morphology is the special study that, perhaps unwittingly, does most to harmonise the divers activities of Natural History, Economic Biology and Academic Science. Cytology, which regards herself as a self-contained study, is nevertheless a branch of Morphology which plays a part in assisting all three. Natural History relies on Cytology for the philosophical knowledge on which her most fundamental systematic conceptions are based. Vegetable Technology is equally beholden to Cytology for information which enables industrial interests to overcome practical difficulties. Cytology deserves the credit of despising an antiquated opprobrium which Academic Science is prone to dread. No study can be so "learned"; the Cytologist assures us that explanations of natural effects offered by Natural History, Academic Science or Economic Biology can only be accepted when confirmed by cytological methods. No study has been more "curious"; Cytology not only explains what the structures connected with nutritive and reproductive functions do, but ventures to tell us how they act.

A generation ago both history and economy were inclined to regard natural knowledge of the characters and virtues of organised things and natural knowledge of organic structure and function as being different in kind. A corollary was the tendency to narrow the incidence of the term Economic Biology to studies connected with the application of functional and structural knowledge. This tendency has disappeared with the idea that underlay it. Even Academic Science, under the consolidating stress of conflict, so far discarded prepossession as to vie with Natural History and Economic Biology in devoting her energies to the task of applying natural knowledge at first hand.

This happy concurrence of the agencies concerned with the improvement of natural knowledge restored a philosophical conception, evolved two and a half centuries ago and kept alive by Natural History, through good report and ill, ever since. It also restored the practical attitude towards Economic Biology adopted by early man. He was sufficiently interested, from primitive times, in the effects of organic

things and in their nutritive and reproductive functions, to be guided by his knowledge as to what he might use and how he should mate. Poetic faculty led him "to project himself into nature" in search of an explanation of the causes of things. While still a hunter he was guided by experience to devise codes regulating the breeding and selection of his own kind. Later, as a herdsman, he extended his interest to include animal-nutrition and animal-breeding. Later still, as a tiller of the soil, this interest embraced plant-nutrition and plant-selection.

When poetic intuition was replaced by divine inspiration, practical expedient yielded to priestly ordinance. The interest in breeding and selection, cankered at the root by Essene doctrine, found an imperfect outlet in "domestication."

Two hundred years ago the subject of plant-nutrition was taken in hand by philosophy. But the study was referred to "statics" and the opportunity was not improved. A century later, the study was resumed, but its improvement was again postponed in deference to the intelligible respect entertained by husbandry for chemistry. It was not till some half a century ago that the philosophical investigation of plant-nutrition at last came into its kingdom.

The earliest attempt at the philosophical study of reproductive-function was practically contemporaneous with this third happy effort to treat nutritive-function on philosophical lines. But Genetic study was then less fortunate than its cognate and its results were overlooked by Academic Science.

Within our generation that study has been energetically resumed. Imbued throughout with the Natural History spirit, it has placed practical animal-breeding on a philosophical basis and has made economic plant-breeding a possibility. The immediate value of the results to Economic Biology and their potential consequence to Moral Philosophy are already fully understood.

I must apologise for having dwelt at such length on facts that are familiar and relationships that are well appreciated. We Economic Biologists are, we know, but a feeble folk, "hewers of wood and drawers of water" to some of the "sciences" and many of the "arts." But this very circumstance makes us "citizens of no mean city" and it may not be wholly amiss, when taking counsel together, to remind ourselves, sometimes, of the fact.

PROCEEDINGS OF THE ASSOCIATION OF ECONOMIC BIOLOGISTS

February 18th, 1921.

Presidential Address.

Some Relationships of Economic Biology—Sir David Prain.

March 11th, 1921.

EXHIBITS.

I. (*a*) Cultures of *Polyopeus*.

(*b*) Demonstration of the Enzymic Action of *Polyopeus* in Solid Nutrient Media containing Starch—A. S. Horne.

II. (*a*) Some Cultures of the Causal Organism of a Potato Disease.

(*b*) A Novel Method of Inoculation of Potato Tubers—S. G. Paine.

III. The Differentiation of Biological Forms of *Puccinia Graminis Tritici* by the Sorus Characters on Infected Pure Lines of Wheat. (From the Department of Plant Pathology of the University of Minnesota)—Wm. B. Brierley.

IV. Potato Tubers infected simultaneously by Corky Scab and Wart Disease—G. C. Gough.

PAPERS.

I. The Cells of Plant Tissues in Relation to Cell Sap as the Food of Aphids—J. Davidson.

II. Ceylon Ambrosia Beetles and their Relation to Problems of Plant Physiology—E. R. Speyer.

April 22nd, 1921.

PAPERS.

I. Green Plant Matter as a Decoy for *Actinomyces Scabies* in the Soil—W. A. Millard.

II. The Action of Bacteria and Protozoa in conserving the Nitrogen in Sewage—E. H. Richards.

III. On the Methods of Infection of the Apple Canker Fungus—S. R. Wiltshire.

EDITORIAL NOTE

At the General Meeting of the Association of Economic Biologists held in the Imperial College of Science on Feb. 18th the Treasurer reported that the cost of publication of Vol. VII of *The Annals of Applied Biology* had been so great as to make material financial retrenchment a necessity. The Publications Committee therefore put to the meeting the following proposals which were carried unanimously.

(1) Reduction in size of papers.

All papers published in the *Annals* must be condensed to the utmost and for this year no paper will be accepted exceeding 8000 words in length.

(2) Tabular Matter.

As the printing of tables is a costly process all contributors are requested to reduce tabular matter to the absolute minimum, and if possible to arrange the data in line or graphic form.

(3) Plates and Text-Figures.

All plates must be paid for by the contributors and as regards the text-figures a limited number will be allowed by the Publications Committee.

(4) Reduction in Number of Pages.

Fewer papers will be published in Vol. VIII, each part being limited to 50 pages, with discretion to call an individual part a double number. As far as possible each part will contain papers dealing with different aspects of economic biology.

The above restrictions apply only to papers published at the expense of the Association and not necessarily to those papers for which "grants in aid" have been received.

It is hoped that the reductions outlined above will only be operative for the current year since it is anticipated that in 1922 it will be possible to revert to improved conditions.

All contributors are asked to read carefully the instructions on the back cover of the *Annals*.